BIOAVAILABILITY OF PESTICIDES FOR BIOTRANSFORMATION IN SOILS

By

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CHAPTER 1

INTRODUCTION

Ground water, due to its quality and availability, is an important source of water in the United States. In fact, 53% of the total U.S. population uses it as the source of drinking water. In the rural sections of the country, 97% of the households rely on ground water (Moody 1990). One problem associated with our heavy reliance on ground water is that it is becoming contaminated.

A major source of ground water contamination is farming activity. Through inefficiencies in the application of fertilizers and pesticides, the ground water is being polluted by nitrates and pesticide residues (Hallberg 1987). On top of this, the use of pesticides is increasing. Its usage has increased 250% since 1964 (Newman 1993). Currently, more than 800 million pounds of pesticides are used in the U.S. annually and of that amount, 66% are herbicides (Newman 1993).

As late as the end of the 1970's, many scientists believed that pesticides could only reach the ground water in locations that contained cracked or coarse soils, shallow ground water tables, or high pesticide application rates (Bouwer 1989). Today, much to our chagrin, this has been proven false. Now, with many more wells being tested, pesticides are being found more frequently. For example, Hallberg (1987) reported that 17

pesticides were found in the ground water of 23 states. In 1989 Bouwer reported that the EPA detected 46 different pesticides in 26 states. In 1990, Moody reported that pesticides have been discovered in the ground water of 44 states. Finally, in 1992, Holden and coworkers conducted a well survey in a known pesticide usage area. This usage area contained 6 million private wells serving 20 million people. They found the pesticides alachlor, metolachlor, and simazine occurred in about 1% (or 60,000) wells. The pesticide atrazine occurred in about 12% (or 720,000) wells. Also, scientists are concerned that as well monitoring increases, there will be an increase in the number and concentration of the pesticides being detected in ground water (Moody 1990).

The problem with the presence of pesticides in ground water is their effect on human health. Scientists have proven an association between physical contact with pesticides and various health problems, including cancer, male sterility, birth defects, and nervous system disorders (Bouwer 1989). Two commonly used pre-emergent herbicides, which have been found in Oklahoma's ground waters, are alachlor {2- chloro-2',6'-diethyl-N-(methoxymethyl)-acetanilide} and propachlor {2-chloro-N-isopropyl-N-acetanilide} (NAS 1977). Toxicology studies using rats, have shown that alachlor is carcinogenic, "causing benign and malignant tumors of the nasal turbinate, malignant stomach tumors, and benign thyroid tumors" (WHO 1993). Propachlor was reported to cause ". . . dystrophic changes in the liver and kidneys of rats, mice, and rabbits" (NAS 1977). The EPA set the maximum contaminant level for alachlor at two µg/L and propachlor is listed as a contaminant to be monitored (Pontius 1992).

Research Objectives

Using alachlor and propachlor, this research project will attempt to answer several specific questions about the fate of these pesticides in ground water. First, it is known that sorption makes pesticides unavailable to bacteria. To find out if bioremediation of contaminated soils and ground waters is a viable remediation technique, the extent that sorption makes pesticides unavailable to bacteria needs to be determined. Second, it is also the intention of this research project to provide more information about how electron acceptor condition and the presence of outside carbon sources affect bioremediation. The specific objectives of this study are listed below:

1. Evaluate the biotransformation of the herbicides alachlor and propachlor in soil-water systems under aerobic and sulfate-reducing conditions.
2. Describe the effect of sorption on these systems and evaluate its impact on the availability of pesticides for biotransformation.
3. Further investigate the effect of acetate as an added carbon source on such systems.

CHAPTER II

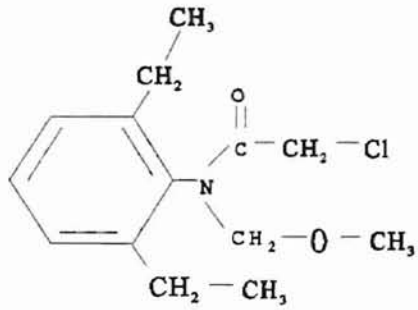
LITERATURE REVIEW

As noted, herbicide contamination of ground water is a wide spread problem. A remediation technology that is showing promise in its ability to clean up pesticide contamination is bioremediation. The topics covered in this chapter are bioremediation, the effect of acetate and electron acceptors on bioremediation, and how adsorption effects bioavailability.

Bioremediation

Bioremediation is an important process affecting the acetanilide herbicides in soil (Villarreal 1991). Field and laboratory studies have proven that alachlor and propachlor (displayed in figures 1 and 2) can be biotransformed. In 1974, Beestman and Deming conducted laboratory and field studies on the degradation of the acetanilide herbicides propachlor, alachlor, and butachlor. They found that microbial decomposition follows first-order kinetics and is primarily responsible for the degradative removal of the pesticides from soils. Under the same conditions, the acetanilide herbicides were 50 times more stable in sterile soil than they were in a "live" soil. The half lives of propachlor and alachlor, in sterile soil, were 146 and 469 days, respectively. The half life of propachlor

Alachlor



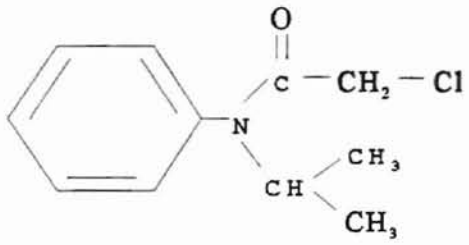
2-chloro-2', 6'-diethyl-N-(methoxymethyl)-acetanilide

Properties

Chemical Formula	$C_{14}H_{20}NO_2Cl$
Molecular Weight	269.77
Physical State	white, odorless, crystalline solid
Pk_a	1.62 -2.30
Density (20°C)	1.113
Vapor Pressure (25 °C)	1.65 x 10-5 mm Hg
Water Solubility (22 °C)	240 mg/l
Boiling Point (°C)	100 (at 0.003 mm Hg)
Melting Point (°C)	40

Figure 1. Properties and Structure of Alachlor (Ware 1988)

Propachlor



2-chloro-N-isopropylacetanilide

Properties

Chemical Formula	$C_{11}H_{14}ClNO$
Molecular Weight	211.1
Physical State	Tan, solid
Pk_a	1.62-2.30
Vapor Pressure (25 °C)	7.73 x 10-4 mm Hg
Water Solubility (20 °C)	580 mg/l
Boiling Point (°C)	110 at 0.03 mm Hg
Melting Point (°C)	77

Figure 2. Properties and Structure of Propachlor (WHO 1993)

and alachlor in live soil were 4.4 and 7.8 days, respectively. They also found that propachlor was not as persistent as alachlor, which is indicated by the length of their half lives. Zimdahl and Clark (1982) observed similar properties for these herbicides. They also found that the water content and the soil temperature affected the biodegradation rate of the pesticides. The greater the temperature and water content, the faster the herbicides were degraded. Again, the degradation rate was first-order. The half lives of the pesticides were a little longer than those reported by Beestman and Deming (1974), but showed the same trend of persistence between the herbicides. The half life of alachlor was between 9 and 11 days and the half life of propachlor is from 4 to 7 days. Other researchers, such as Walker and Brown (1985) have had similar results.

Degradation rates are also affected by soil conditions and properties (Yen et al. 1994). Soil properties not only change with soil type, but they also change with soil depth. Yen et al. (1994) tested biodegradation in four different soils and at various depths within those soils. The length of the half life of alachlor depended upon the type and the depth of the soil. The results are listed in Table 1. According to Walker and Brown (1985), the decrease in degradation with depth may be due to the lack of the subsoil's previous exposure to the pesticide. Another group of researchers, Pothuluri et al. (1985), hypothesizes that the reason for the decrease in degradation with an increase in soil depth is due to the decrease in nutrient availability for the cometabolism of the herbicides.

Table 1. Half life of alachlor degradation as a function of soil type and depth

From Yen et. al (1994)

Soil Type	Depth (cm)	Half life (days)
Kim (clay loam)	0-15	31
	30-45	28
	60-75	63
Port Byron (silt loam)	0-15	44
	30-45	56
	60-75	72
Webster (silty clay loam)	0-15	66
	30-45	37
	60-75	73
Estherville (sandy loam)	0-13	61
	25-36	55
	46-56	54

Effect of Acetate on Biotransformation

Cometabolism is microbial conversion of substrates to organic products when growth is supported by another compound. Microorganisms do not obtain energy from the conversion nor do they obtain carbon that can be used for biosynthesis (Novick and Alexander 1985). Researchers (Novick and Alexander 1985; Pothuluri et al.1990; Yen et al.1994) have found that both alachlor and propachlor are degraded by a cometabolic process. Novick and Alexander (1985) found that microbial populations were not able to mineralize alachlor or propachlor in six weeks. They did find organic products of the compounds, indicating that the compounds were being cometabolized. They also found

that the rate of cometabolism increased proportionally with an increase in the substrate concentration. Yen et al. (1994) determined that the process was cometabolic due to the by-products from the metabolic reaction. Using ^{14}C-labeled alachlor, they found none of the carbonyl-labeled alachlor was mineralized to CO_2. The principal nonpolar metabolite was 2,6-diethylaniline. Finding the principal metabolite to be an organic product indicates microbial cometabolism.

How susceptible a compound is to microbial degradation can be affected negatively by a low concentration of the compound or a limitation in the availability of other carbon sources. Carbon sources, such as glucose, are able to support the growth of bacteria that can cometabolize propachlor and alachlor (Novick and Alexander 1985). A theory, based on theoretical hypothesis and empirical observation, on how limited carbon sources effect biodegradation has been suggested by several researchers (Rittmann et al. 1980; McCarty et al.1981; Schmidt and Alexander 1985). They suggest that there exists a primary growth substrate concentration threshold below which a steady state concentration cannot be less than, since at this substrate concentration the rate of cell decay would be greater than the cell growth. The threshold concentration is a function of the bacteria's growth and decay kinetics. Primary substrates are compounds above the threshold level that contribute energy and carbon for biosynthesis. They can be used to increase the rate of cometabolism merely by increasing the number of cells able to perform cometabolism (Novick and Alexander 1985) . Secondary substrates are compounds below the threshold level that do not contribute enough energy and carbon for biosynthesis (Namkung and Rittmann 1986).

A wide variety of compounds can be used by various microbial cultures as a primary substrate. Schmidt and coworkers (1985) reported that some easily degraded substrates and uncharacterized organic compounds can play an important role in the degradation of organic compounds at low concentrations. Novick and Alexander (1985) used glucose and aniline as primary substrates in the degradation of propachlor. They found that glucose doubled the metabolism of propachlor, while aniline did not have any effect on the metabolism of propachlor. Bouwer and McCarty (1983) tested acetate as a primary substrate, and they found that chlorinated benzenes could be used as secondary substrates under aerobic conditions. Garrett (1993) tested acetate as a primary substrate in the microbial degradation of alachlor and propachlor. He found a greater rate of degradation when acetate was being fed to the bacteria than when the pesticides were being fed alone. Wang (1994) also used acetate as a primary substrate in the degradation of propachlor and alachlor. He also found that there was a greater rate of degradation when acetate was being fed to the bacteria.

Effects of Electron Acceptors on Biotransformation

The electron acceptor used by microorganisms is an important factor in biotransformation. The electron acceptor is used for deriving energy from the electron donor. Microbes use the electron acceptors that provide the most free energy during respiration (Cobb and Bower 1991). The common electron acceptors are oxygen, nitrate, Mn(IV), Fe(III), sulfate, and carbon dioxide. Oxygen provides the most free energy per mole, with the free energy decreasing further down the given list (Cobb and Bower 1991).

Of the electron acceptors on this list, the two used for the research project are oxygen and sulfate.

Oxygen

Pothuluri et al. (1990) studied biodegradation in the soil profile. They generally found that alachlor and propachlor degraded more quickly under aerobic conditions than anaerobic conditions. They also noticed that the rate of degradation decreases with the depth of the sample origin, indicating that the soils were nutrient limited. They tested this hypotheses and found that when they added nutrients the degradation rate in the subsoils and one of the aquifer soils increased. Under aerobic conditions, Garrett (1993) found that in the presence of acetate, alachlor was more susceptible to biotransformation than propachlor. Without acetate, propachlor was biotransformed at a greater rate than alachlor. Another researcher, Wang (1994), found that in the presence of acetate, propachlor was aerobically biotransformed at a faster rate than alachlor. When oxygen was removed, the rate of biotransformation decreased but did not stop. Biotransformation continued without acetate and propachlor continued to be biotransformed more than alachlor. He stated that the reason that the biotransformation continued was that the culture was in an endogenous phase.

Sulfate

Wang (1994) found that propachlor is biotransformed at a faster rate than alachlor in sulfate-reducing conditions. He also found that these pesticides are biotransformed to the greatest degree in sulfate-reducing conditions. Wilber and Parkin (1995), while only using alachlor, had similar results. Alachlor is biotransformed at a faster rate in sulfate-

reducing conditions than aerobic conditions.

A side reaction needs to be considered under sulfate-reducing conditions. Previous work has identified the reaction of bisulfide, HS^-, which is produced under sulfate-reducing conditions, with numerous chlorinated aliphatic compounds. Garrett (1993) found that both alachlor and propachlor react with the bisulfide ion and propachlor reacts at a faster rate than alachlor. Garrett found that propachlor has a pseudo-first order reaction rate constant of 0.0028/h-mg/L $[HS^-]$which can be mathematically expressed as follows:

$\ln(C/Co) = 0.0028\ [HS^-]\ t$ . . . . . [1]

C/Co = fraction remaining of pesticides

$[HS^-]$ = bisulfide concentration, mg/l

t = reaction time, hour

Garrett also found that alachlor has a pseudo-first order reaction constant of 0.0011/h-mg/L $[HS^-]$ that can be mathematically expressed as follows:

$\ln(C/Co) = 0.0011\ [HS^-]\ t$ . . . . . [2]

Wang (1994) determined that when low bisulfide concentrations are present, the bisulfide reaction, when compared to the biotransformation of the pesticides, is relatively minor.

Adsorption

Primarily, there are seven different ways that the "life expectancy" of pesticides in soil are affected: movement, volatilization, chemical decomposition, photochemical decomposition, plant uptake, microbial decomposition, and adsorption (Baily 1970).

Adsorption directly affects the other six, including microbial decomposition. Zimdahl (1982) stated that the microbial decomposition rate decreases as adsorption increases. Researchers have noticed that several organic compounds and pesticides such as parathion, 1,2-dibromoethane (EDB) and dibromochloropropane (DBCP) have been able to persist in soils for years despite their ability to be biodegraded by bacteria found in soils (Wolfe et al. 1973; Buxton and Green 1987; Steinberg et al. 1987). Recent research has shown that organic contaminants that are bound to soils are unavailable for biological degradation (Steen et al. 1980; Shimp and Young 1988; Ogram et al. 1987). Steinberg et al. (1987) used EDB in their experiments. They found that aged EDB was unyielding to biodegradation whereas newly applied EDB was readily biodegraded. They suggested that the aged residues occupied remote sites in the soils, accounting for their extremely slow release times and their increased persistence. Another group of researchers, Scribner et al. (1992), used the herbicide simazine in their experiments. They determined that aged simazine is resistant to desorption into the aqueous solution and thus to degradation by indigenous soil bacteria. However, newly applied simazine was rapidly desorbed and degraded by the indigenous soil microbes. They suggested that the aged simizine is partitioned deeply in organic material or entrapped in soil micropores which is inaccessible to microbial degradation.

Since adsorption has such an effect, it seems to be the major factor controlling the fate of pesticides in soil. The mechanism and amount of adsorption is affected by the physical-chemical nature of the pesticide along with soil properties. These will be discussed below.

Physical-chemical nature

Significant properties of the acetanilide herbicides, which include alachlor and propachlor, are low-to-moderate volatility, moderate water solubility, moderately low polarity, and non-ionizable functional groups (Webber 1982, Locke 1992). The adsorption of herbicides by soils can be related to the water solubility of non-ionizable chemicals (Webber 1982). Non-ionizable compounds with higher water solubilities are adsorbed to organic matter to a lesser extent than chemicals with lower water solubilities. A water solubility example using compounds from the acetanilide family follows. Alachlor has a water solubility of 242 ppm and metolachlor has a water solubility of 530 ppm. Alachlor has a higher affinity for organic matter than metolachlor, and this higher affinity is attributable to the difference in water solubilities (Peter 1985).

Soil

The acetanilide herbicides are adsorbed by both the organic and inorganic constituents in the soil (Webber 1982). The soil properties that effect the adsorption of acetanilide pesticides are organic matter, clay content, surface area, and cation exchange capacity. The last two, surface area and cation exchange capacity, are highly correlated with the soils content of organic matter and clay particles (Webber et al. 1993). Acetanilide herbicides are primarily adsorbed by organic matter instead of clay minerals (Webber 1982). At the present, the accepted theory for the adsorption of the pesticides by soil organic matter is that the non-polar molecules are forced out of the water and onto the organic matter by hydrophobic repulsion. This behavior is most apparent when the

pesticide is neutral, i.e. non-ionic and non-polarizable (Grundl 1993).

Soil organic matter is an important site for acetanilide compounds (Locke 1992). Several different investigators studying acetanilide compounds have shown a high correlation between organic material and adsorption. Using alachlor, Locke (1992) found the greater the amount of organic matter, the greater the adsorption. Other investigators using acetanilide herbicides had the same results. For example, Wood et al. (1987) used metolachlor and Sato et al. (1987) used butachlor, and both observed similar relationships.

One way to quantify the sorption of herbicides by organic matter is the use of the K_{oc} value. The K_{oc} values characterizes herbicide partitioning between the aqueous solution and the organic phase in soil (Locke 1992). Locke determined K_{oc} by using the Freundlich equation:

$$x/m = K_{oc}C_s^{(1/n)} \quad [3]$$

where x/m represents the sorbed herbicide (μmol/kg), and

C_s is the concentration of the herbicide in solution (μmol/l), and

1/n is the Freundlich exponent.

A range of K_{oc} values for alachlor have been reported in the literature. Donigan (1987) reported a K_{oc} value of 342 ml/g. Pereira (1990) reported 170 ml/g. In 1992, Locke reported a value of 298 ml/g. No K_{oc} values for propachlor were found in the literature.

If the weight ratio between the clay portion and the organic portion of the soil is greater than 30, then the sorption contribution from the clay portion of the soil may be significant (Locke 1992). Organic material can affect sorption by clay minerals. If the ratio is less than 30, the organic portion may block the pesticide's access to some clay

sorption sites (Locke 1992). Researchers have shown that organic pollutants are attracted to mineral surfaces (Grundl 1993). In fact, it has been proven that mineral phase sorption is important in organic-poor soils and the extent of the sorption is inversely related to the hydrophobicity of the solute (Grundl 1993). Several theories exist on the interaction between organics that contain polar groups and clays. The theories involve either ionization of the organic pollutant and sorption, some form of hydrogen bonding, or Van der Walls' forces (Grundl, 1993). Due to their high solubilities and polar functional groups, the acetamide (or acetanilide) herbicides are theorized to be adsorbed to the minerals surfaces by polar attractive forces (Grundl, 1993).

As with organic sorption, one way to quantify the sorption of herbicides by the mineral content is the use of the K_m value. The K_m values characterize the separation of the pesticide between the aqueous solution and the clay mineral phase in the soil (Grundl, 1993), and as such it is analogous to K_{oc}, described previously. Alachlor has a reported K_m value of 5 ml/g (Grundl, 1993). No values for propachlor found in the literature.

Adsorption and Bioavailability Studies

As stated earlier, as adsorption increases the rate of biodegradation decreases. A method that has been used successfully to study adsorption involves biofilm column studies. Biofilm column studies have been used by researchers for years (Bouwer and McCarty 1985; Miller et al. 1985; Lanzarone and McCarty 1990). In studies that used columns filled with aquifer material, the interpretation of results was complicated by compound adsorption (Siegrist and McCarty 1987). Siegrist and McCarty (1987) studied

a halogenated aliphatic compound, trichloroethane (TCA) which is adsorbed by aquifer material, and devised a column method for determining sorption and biotransformation by using mass balances. They determined the mass fed to the column, the mass sorbed to the aquifer material, and the mass in the column effluent. They made the mass balance comparing the total effluent mass of TCA removed, mass effluent of DCA (a by-product of the biodegradation of TCA) and the estimated masses of TCA and DCA sorbed to the aquifer material. Using their mass balance method, they concluded that the DCA was twice as strongly sorbed to the aquifer material as the TCA. Similar studies using pesticides were not fund in literature.

Summary

A review of some of the current literature concerning alachlor and propachlor demonstrate that progress has been made in understanding their fate and transport. It is known that alachlor and propachlor can be biotransformed and, under most conditions, adsorbed to soils. However, how adsorption affects the amount or rate of the transformation is not as clear. Few of these earlier studies specifically address the effects of adsorption on the biotransformation of alachlor and propachlor. As such a number of important questions remain. For example, what is sorption's impact on the bioavailability of pesticides for biotransformation? What are the effects of acetate as an added carbon source on such systems? Furthermore, what are the rates of biotransformation of the

herbicides alachlor and propachlor? The current literature provides the basis for selecting objectives of this study in an attempt to better understand how adsorption affects the biotransformation of alachlor and propachlor.

CHAPTER III

MATERIALS AND METHODS

The following chapter describes the materials used and the methods employed in this research.

Chemicals

Most of the chemicals used in this project are commercially available from Fisher Scientific and were used without any further purification. Methanol and ethyl acetate were HPLC-grade solvents or better. The aqueous stock solutions of alachlor and propachlor were prepared from analytical-grade chemicals purchased from Chem. Service, (West Chester, PA).

Pesticide Analysis

Pesticide analyses were conducted on two different mediums, liquid effluent and soil samples from the columns. The liquid effluent pesticide concentrations were measured by the solid phase extraction method described by Thurman et al. (1990). Prep Sep. C_{18} Cartridges (Fisher Scientific, Fair Lawn, NJ) containing 360 mg of 40 μm C_{18} bonded silica were used. The C_{18} cartridges were prepared by washing with, in order, 2 mL of methanol, 2 ml of ethyl acetate, 2 mL of methanol, and 2 ml of distilled water. The

column effluent sample (25 ml) was passed through the Prep Sep. cartridge, at an approximate rate of seven ml/minute, using a Prep Torr Vacuum Box (Fisher Scientific, Fair Lawn, NJ). The cartridge was dried with air to remove residual water and then eluted with 2.0 ml of ethyl acetate.

Two different soil extraction methods were used. The first method was described by Huang and Pignatello (1990), and was selected for several reasons. First, they used metolachlor and atrazine in their experiments. Metolachlor is in the same chemical family as alachlor and propachlor. Second, when methanol was used as the solvent, this method was found to give high extraction rates and yield chromatograms with less noise than other water-miscible solvents. For example, they claimed an 86% recovery for metolachlor. They did not mention a recovery for atrazine. In using this method, approximately 70 grams of soil were combined with 75 ml of solvent in a 250-ml Erlenmeyer flask. The solvent was a mixture of 80% methanol and 20% deionized water, by volume. Samples were agitated at room temperature on a shaker table for 24 hours. After agitation, 10 ml of the supernatant was mixed with 50 ml of deionized water and 10 ml of methyl chloride in a separatory funnel. The separatory funnel was vigorously shaken twice to mix the contents. The methyl chloride was drained from the separatory funnel, put in a round bottomed flask and evaporated in a steam bath. To redissolve the pesticide that collected on the surface of the flask, 2.1 ml of ethyl acetate was pipetted into the flask and then swirled around until it touched all the surfaces of the flask. The ethyl acetate was allowed to evaporate until only 2 ml was left and then was pipetted into a test tube.

The second method is given by Guo et al. (1993). This method was selected for

a couple of reasons. First, they also used alachlor in their experiments. Second, this method yielded a 73.4 ± 5.2% recovery for alachlor. In using this method, approximately 70 grams of soil were combined with 70 ml of solvent in a 250-ml Erlenmeyer flask. The solvent was a 6:1 mixture, by volume, of ethyl acetate and deionized water. Samples were agitated at room temperature for 24 hours on a shaker table. After the samples were shaken, the supernatant was poured into a 250-ml flask. The soil was further rinsed twice with 25 ml of ethyl acetate. The extracts were combined in a flask and evaporated in a steam bath. 2.5 ml of ethyl acetate was added to the flask and swirled around until it touched all the surfaces of the flask. The ethyl acetate was allowed to evaporate until there was only 2 ml left and was then pipetted into a test tube.

The ethyl acetate samples, from both liquid and soil extractions, were analyzed by gas chromatography (GC). The extracts were injected (2 µl) onto a DB-5 stationary phase fused silica capillary column (film thickness, 0.25 µm; inner diameter, 0.25 mm; length 30 m; J&W Scientific, Folsom, CA) in a model 5890 Hewlett-Packard Series II GC equipped with an electron capture detector (ECD). Quantification was achieved by injecting standards, treated like samples, and comparing relative areas under separated peaks recorded by a model 3396 Hewlett Packard Series II integrator. Injections were made in the split mode (ratio 45:1) at an injector temperature of 200 °C and a column temperature of 195 °C. The carrier gas was helium with a flow rate of 1.7 ml/min at a column head pressure of 13 psi. The ECD make up gas was a 95% argon; 5% methane mixture.

Anionic Compound Analysis

Acetate and sulfate were analyzed by ion chromatography (Dionex series 2000i/sp ion chromatograph (IC) module) as described by APHA (1989). Aqueous standards for each ion were prepared gravimetrically in a range from 5 to 120 mg/l. The standards were used to calibrate the instrument during analysis. Samples (0.2 ml) were collected from either reactor influent or effluent. Before injection, the samples were filtered by a 0.2 μm syringe filter (Cole-Parmer Instrument Co., Chicago IL). The IC eluant consisted of a mixture containing 0.0017M $NaHCO_3$ and 0.0018M Na_2CO_3. Detection was accomplished by using an electrical conductivity detector with anion suppression.

Column Reactors

A total of eight columns were used in this project. For a breakdown see table 2. Four of the columns were fed oxygen as an electron acceptor, two used sulfate as an electron acceptor, and the last two were sterile. The columns were operated for about nine months to evaluate biodegradation and adsorption of alachlor and propachlor.

The aquifer material used to fill the columns originated in a site next to the Norman, Oklahoma municipal landfill. The site, described by Beeman and Suflita (1987), is a shallow unconfined alluvial sand aquifer. The aquifer is typically anoxic with mostly sulfate-reducing and methanogenic bacteria. Aquifer sediments were manually collected after opening a small trench with a backhoe. The samples were collected approximately

Table 2. Columns Used in the Study

	Column Name	Electron Condition
Column 1	Sterile Column 1	Aerobic
Column 2	Sterile Column 2	Aerobic
Column 3	Aerobic Column 1	Aerobic
Column 4	Aerobic Column 2	Aerobic
Column 5	Aerobic Column 3	Aerobic
Column 6	Aerobic Column 4	Aerobic
Column 7	Sulfate Reducing Column 1	Sulfate Reducing
Column 8	Sulfate Reducing Column 2	Sulfate Reducing

two feet below the water table that lies about four feet below the ground surface.

The columns (see Figure 3) and the column operations are similar to the method described by Siegrist and McCarty (1987). The glass columns used are 45 cm in length and 40 mm in diameter. They were filled by adding one hundred ml of deionized water to the columns. Aquifer material was then added with a spoon through the top of the columns. The columns were tapped, with a glass rod, as they were being filled to get a good settling of the aquifer material. This method allowed for good flow conditions in the columns, although some fine particle fractions were lost by this procedure. The columns were visually inspected for uniformity of packing, then covered with aluminum foil to prevent the growth of photosynthetic organisms. At this point, the Sterile Columns were autoclaved for two hours in an effort to kill all the organisms in the soil. All of the columns were kept fully saturated and at room temperature (approximately 21 °C) for the

duration of the experiment. All the tubing and fittings on the columns, except the stoppers, were made of either Teflon or silicone to reduce sorption.

Bromide tracer studies were conducted on the columns to investigate the flow characteristics within the columns for several reasons. First, the volume of column influent had to be larger than the total column pore volume to displace the column fluids completely. Second, it was important to determine the sample size so that samples taken for analysis were not affected by diffusion of the new influent feed due to mixing. The step feed method was used to conduct the bromide tracer study. The volume of tracer used was greater than the pore volume. Samples were taken until the concentration of the tracer effluent was approximately the same as the influent. The breakthrough curves for the bromide tracer studies are in Appendix A. Based on these curves, the first 25 ml of effluent from the column was found to accurately represent what was in the column and was not contaminated by the new feed. Seventy-five ml is the volume of feed required to displace the column pore volumes completely.

The column fluids were exchanged by peristaltic (Masterflex) pumps in the upflow mode. The influent feed was pumped through the bottom of the column and the effluent was collected from the top of the column. For the first couple of months the feed was exchanged every 48 hours, then the feeding cycle was changed to 96 hours.

Column Feed

The column feed solution was designed to resemble typical ground water (Freeze and Cherry 1979). The constituents of the feed solution are listed in Table 3. In the

aerobic columns, the target acetate concentration in the first and second periods of the experiment was 36 mg/l and 50 mg/l, respectively. These concentrations are slightly greater than the stoichiometric amount needed to deplete the dissolved oxygen levels in the aerobic columns.

Table 3. Column Feed Composition

Constituents	**Sterile**	**Aerobic**	**Sulfate Reducing**
Primary Substrate Acetate		50 mg/L	80 mg/L
Electron Acceptor	8.0-9.0 mg/L DO	8.0-9.0 mg/L DO	120 mg/L Sulfate
Secondary Substrate	300 µg/L Alachlor 300 µg/L Propachlor		
Inorganic Nutrients			
K_2CO_3	27.6 mg/L	27.6 mg/L	55.2 mg/L
$MgCl_2.6H_2O$	16.3 mg/L	16.3 mg/L	32.6 mg/L
$CaCl_2.2H_2O$	11.8 mg/L	11.8 mg/L	23.6 mg/L
$FeCl_2.4H_2O$			1.4 mg/L
$CoCl.4H_2O$			0.05 mg/L
$ZnCl_2$			0.007 mg/L
H_3BO_3			0.007 mg/L
$MnCl_2.4H_2O$			0.06 mg/L
NH_4Cl			0.4 mg/L
$NiCl_2.2H_2O$			0.01 mg/L
KI			0.05 mg/L
NH_4HCO_3	0.4 mg/L	0.4 mg/L	
Buffer pH=7.1			
KH_2PO_4	2.1 mg/L	2.1 mg/L	26.2 mg/L
K_2HPO_4			40.0 mg/L
Na_2PO_4			21.4 mg/L
$NaHCO_3$	3.3 mg/L	3.3 mg/L	

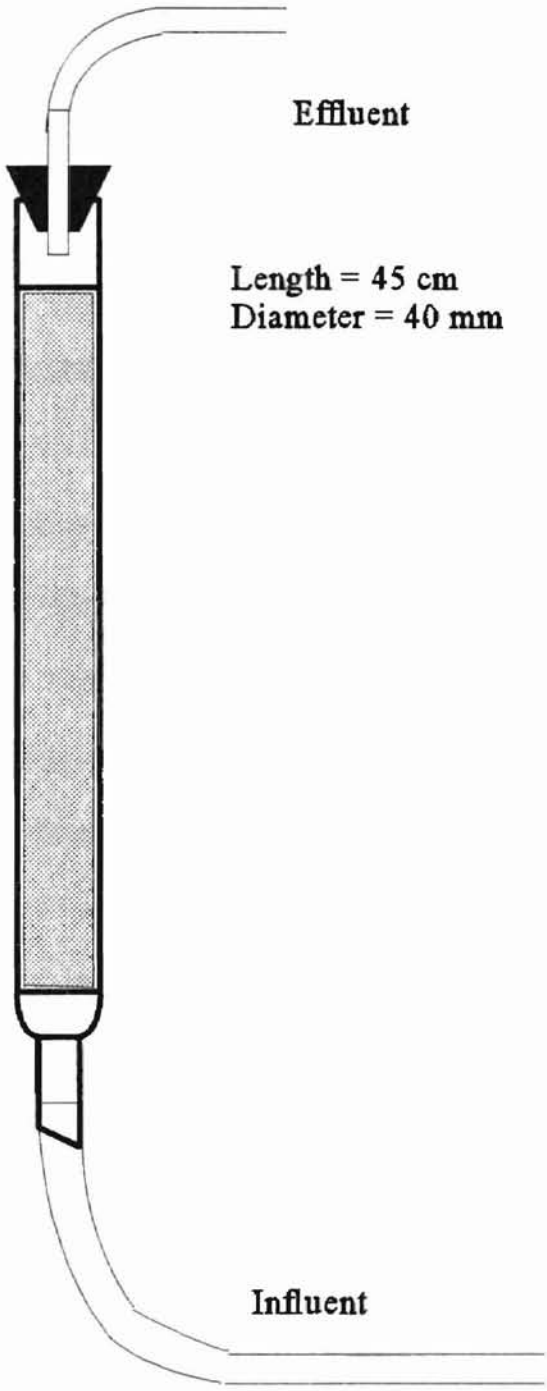


Figure 3. Schematic Diagram of Column Reactors

CHAPTER IV

RESULTS AND DISCUSSION

This project used a total of eight column reactors. The two sterile columns were operated from March 2, 1995 to October 12, 1995. They were intended for use as controls to measure adsorption without biodegradation. Four aerobic columns were operated from February 1, 1995 to September 25, 1995 to evaluate the biotransformation and adsorption of alachlor and propachlor under aerobic conditions. Two sulfate-reducing columns were operated from May 27, 1995 to September 25, 1995 to measure the amount of biotransformation that occurs under sulfate-reducing conditions.

The pesticide effluent concentrations were monitored to examine their biotransformation and the acetate effluent concentrations were measured to check for the presence of acetate-utilizing bacteria. The data for similar columns was combined in the data analysis. The effluent concentration data of these constituents appear in Appendixes B and C. The influent pesticide concentrations were measured and compared with the effluent concentrations to check for the occurrence of adsorption and biotransformation. Influent concentrations appear in Appendix E.

Sterile Columns

The purpose of the sterile columns was to investigate the effect of adsorption and its impact on the bioavailability of the pesticides. These columns were operated under two different conditions. The only variable that changed between the two conditions was the feeding time. During the first portion of the experiment, the column feed was exchanged every two days and during the last part of the experiment the feed was exchanged every four days. Exchanging the feed in this paper means to displace all the fluid in the reactor with new feed. This change was made in order to test the influence of contact time on adsorption. The feed solution was the same as that described for the aerobic columns, except the acetate was removed from the feed.

The sterile columns did not work as expected. It was expected that the effluent concentrations would increase and eventually plateau to the influent concentration levels, once adsorption equilibrium had been achieved, but this did not happen. As seen in Figures 4 and 5, the sterile columns were apparently exhibiting signs of biological growth and pesticide biotransformation. An attempt was made to kill the biological growth by applying sodium azide (NaN_3). Sodium azide was first applied to Sterile Column 1 on day 138. The method used was described by Wolf et al. (1989), who suggested using 3.08 mmol of sodium azide per kg of soil. In that study, the sodium azide reduced the bacterial population in three soils and the fungal population in two other soils. The weight of soil in the columns was estimated (280 mg) and the appropriate amount of NaN_3 was dissolved in 75 ml of feed solution and

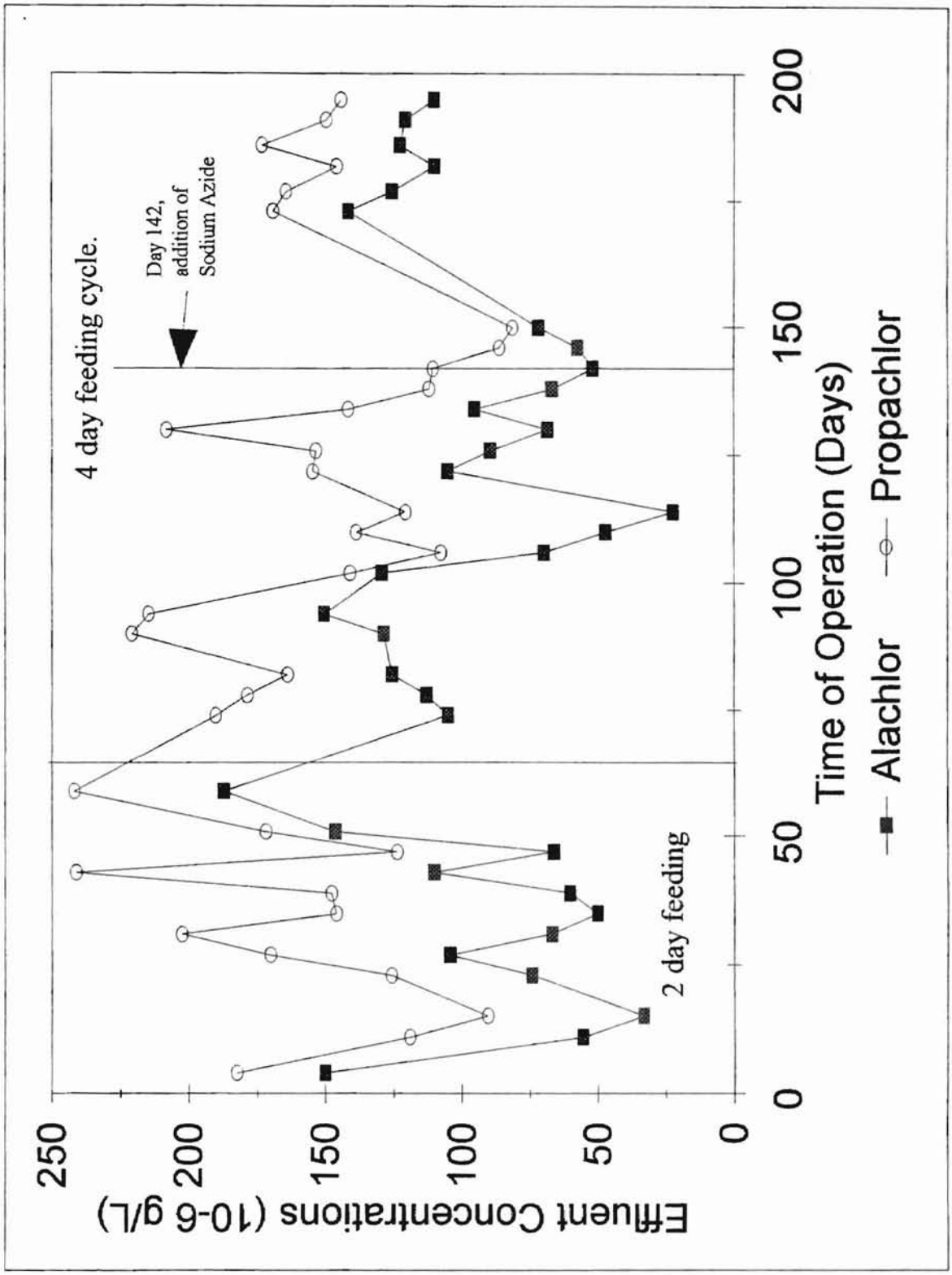


Figure 4. Sterile Column 1

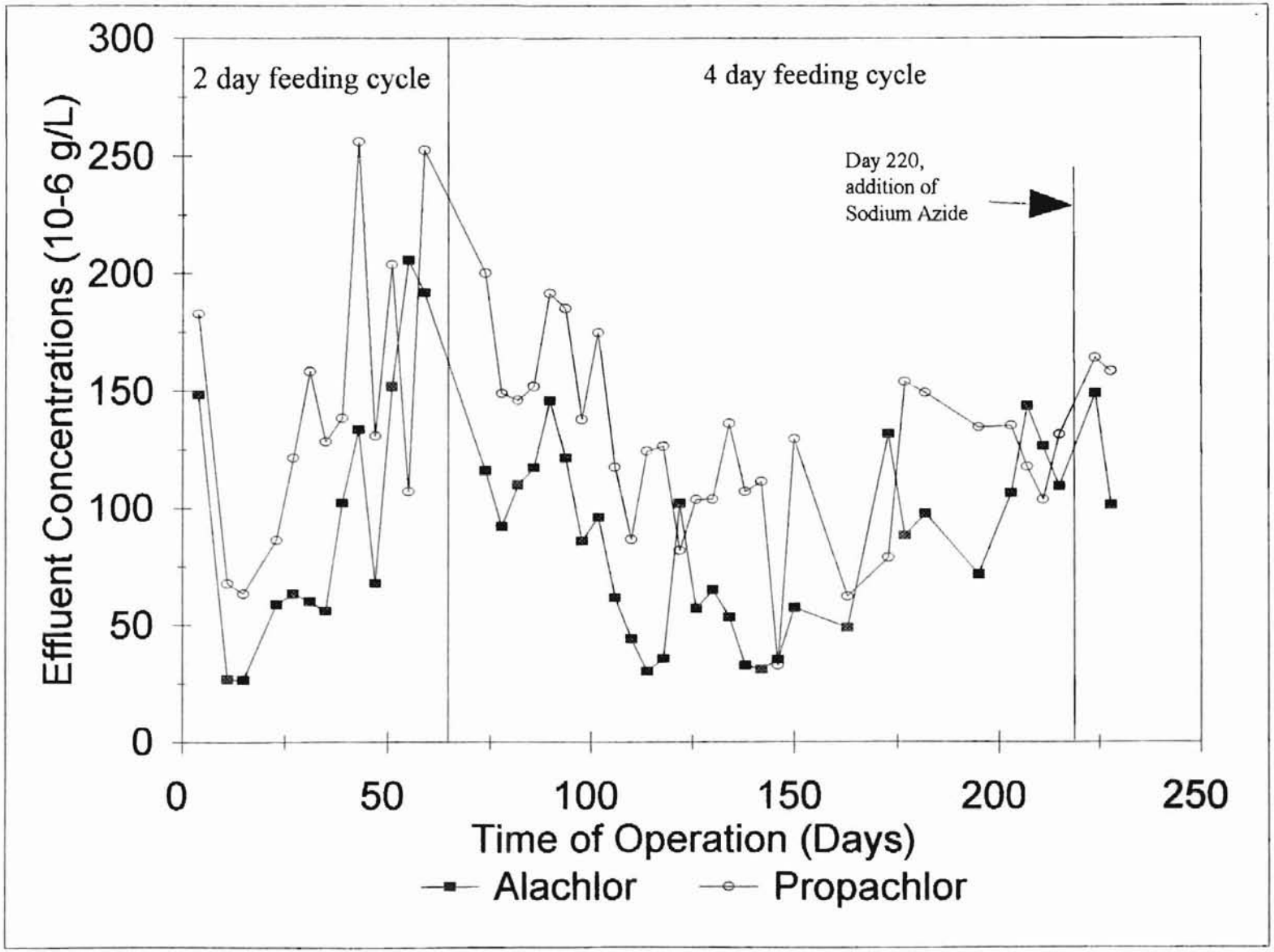


Figure 5. Sterile Column 2

applied to the column. On day 142 the NaN_3-dosed sample was taken and prepared to run on the gas chromatograph (GC). Unfortunately, at this time mechanical difficulties made the GC inoperative for a period of 75 days. However, samples continued to be taken during this period. After the GC was repaired, the day 142 sample was run and it was noticed that the NaN_3 did not significantly affect the results. This result prompted a closer look at the results of Wolf et al.(1989). In this study, air drying was used as the sterilization control. Air drying, depending upon the soil type, produced a bacterial population between 6.43 and 6.99 $\log_{10}$ colony forming units per gram of dry soil. Sodium azide, again depending upon the soil type, produced a bacterial population between 6.07 and 6.33 $\log_{10}$ colony forming units per gram of dry soil. Sodium azide was able to reduce the bacterial population between 56% and 78%, but still left a viable bacterial population. Sterile Column 1 plugged up on day 199, so the second dose of NaN_3 was applied to Sterile Column 2 on day 220. The sodium azide concentration for the second dose was increased by a factor of 10. The sample was taken on day 224 and analyzed. The effluent pesticide level increased, but not dramatically, indicating only a slight effect on microbial activity. Subsequent samples showed a decrease in the effluent pesticide level, suggesting that viable bacteria were still present, and growing, in the column.

In the first portion of the experiment (with a 2 day retention time), the average effluent concentration for propachlor and alachlor was 150.86 µg/l (s.d. 54.7) and 96.07 µg/l (s.d. 54.8), respectively. The difference between the concentration levels is statistically significant (student t-test, at a 95% confidence level) showing the bacteria

preferred alachlor to propachlor. In the second period (With feeding time increased to four days), the average effluent concentration for propachlor and alachlor was 139.94 (s.d. 42.3) and 105.68 (s.d. 37.4), respectively. The difference between these two levels is also statistically significant, showing that the bacteria in the columns still preferred alachlor to propachlor.

The difference between a two day and a four day feeding cycle was also tested, but the differences in the effluent pesticide concentrations for these two feeding times were not statistically significant. There are several possible reasons for the degradation of the pesticides and the lack of a difference between the two and four day feeding cycles. In general, if all of the removal is due to biological activity, the doubling of the retention time should have resulted in increased pesticide removals, assuming equilibrium is established for adsorption. The degradation of the pesticides could have been cometabolic, and as such, the process would continue until the substrate threshold level was reached or until the oxygen was depleted. Recall that no additional organic material was present in the column feed beside the pesticides. However, there are still several possible sources of organic carbon for cometabolism. A small amount of dissolved organic carbon may have been unintentionally present as contaminants of the inorganic salts used in the feed solution, though every precaution was taken to maintain the quality of the feed solutions. A more likely source of dissolved organic carbon is the distilled water. In a study performed on two different types of bacteria by Schmidt and Alexander (1985), the bacteria P. Acidovorans and Pseudomonas sp., placed in inorganic salt solutions made with water that was distilled and then treated with a milli-Q (activated carbon) system,

were able to grow to densities from $1x10^5$ to 5×10^5 and $1.8x10^6$ cells per ml, respectively. Schmidt and Alexander estimate that it would require 90 µg/l phenol to support the same amount of cell growth as in the inorganic salt solution. Doubly distilled water which had been oxidized with $K_2S_2O_8$ still supported the growth of P. Acidiovorans to cell densities between 2×10^4 and $6x10^4$ cells per ml. Hence, since the feed solution in the current study was made with water that was distilled but not treated with the milli-Q system, the amount of biological growth supported by the feed solution might possibly be even greater than Schmidt and Alexander report.

Another possibility is that the bacteria may have been able to directly metabolize the pesticide until the substrate threshold was reached, or until the dissolved oxygen was depleted. Villareal, Turco, and Konopka (1991) were able to identify a metabolic pathway used by bacteria to degrade acylanilide herbicides and were able to isolate six strains of bacteria that were able to degrade propachlor as a sole source of carbon and energy. The metabolism of the pesticides is initiated by aryl acylamidases, enzymes which cleave the pesticide and form an organic acid and an aniline derivative. The bacteria then metabolize the organic acid and leave the aniline derivative alone. Bacteria which mineralize other acylamides with N-alkyl substitutions, i.e., alachlor and metolachlor, have yet to be isolated, but the results of Villareal et al. (1991) indicate they may exist.

A final possibility involves abiotic transformation. Certain organic compounds may be chemically transformed in the presence of mineral surfaces and an oxidant or reductant. For example, King and Reinhard (1994) found that carbon tetrachloride in the presence of a mineral surface (biotite, vermiculite, and/or pyrite) and bisulfide was transformed into

CS_2, CO_2, and $CHCl_3$. Clearly, additional information would be needed to determine if such a reaction was occurring in these reactors. No obvious sign of the presence of sulfide was observed.

Even though the sterile columns exhibited biological activity, making them unusable for controls, several things were still learned from them. First, it is observed that without adding a primary food source to the feed solution, and even following a series of steps to inhibit biological activity, the columns were apparently able to biotransform alachlor and propachlor, and these bacteria preferred alachlor to propachlor. The residence time had little effect on the amount of pesticide degraded, for reasons discussed above. Finally, it must be noted that the organisms that inhabited the columns appeared to be very resistant to sterilization techniques.

Aerobic Column Results

The purpose of these columns was to measure both the extent of adsorption and to evaluate the biotransformation of alachlor and propachlor under aerobic conditions using acetate as a primary carbon and energy source. These columns were run under four different conditions. The first period lasted 59 days, during which the columns were fed every two days. The acetate concentration was 36 mg/l. During the second period, lasting 38 days, the acetate feed was increased to 50 mg/l. The third experimental period lasted 120 days, during which the acetate feed remained constant but the feeding cycle (and hence, the retention time) was increased to four days. The final portion of the experiment lasted 16 days during which the acetate was removed from the influent feed.

The microbial stoichiometric equation for acetate usage under aerobic conditions is as follows (Wang 1994):

$CH_3COO^- + 1.52\ O_2 + 0.096\ NH_4^+ =$

$$0.096\ C_5H_7O_2N + 0.66\ CO_2 + 0.904\ HCO_3^- + 0.86\ H_20 \ldots\ldots\ldots\ldots [4]$$

During the first experimental period, the column feed was exchanged every two days. Effluent acetate concentrations were lower than the influent concentrations by an average of 30 mg/l (s.d.18.5) showing the presence of acetate-utilizing bacteria. The herbicide effluent results are plotted (concentration vs. time) in Figures 6 through 9. The raw data for these figures are in Appendixes B and C. During the first period, alachlor's effluent concentration (average 87.6 µg/l; s.d. 29.7) was lower than propachlor's (average 151.6 µg/l; s.d. 37.5). The difference between these two averages is statistically significant enough to conclude the bacteria in these columns preferred alachlor to propachlor.

During the second part of the experiment, the acetate concentration was increased to 50 mg/l. This had the unexpected result of increasing alachlor's and propachlor's effluent concentrations. The result was unexpected because, in a truly cometablic system, an increase in primary substrate should result in an increase in the bacterial population, which in turn should result in a greater rate of pesticide cometabolism. Alachlor's average concentration increased to 130.8 µg/l (s.d. 33.2) and propachlor's average increased to 169.8 µg/l (s.d. 30.5). Propachlor's effluent concentration was still higher than the alachlor's effluent concentration, but the difference was smaller than in the first period. The difference between the two pesticides is not large enough to conclude, statistically,

that under these conditions the bacteria preferred either pesticide. Also, the difference in propachlor between the first period and the second period is not statistically significant where alachlor's difference is statistically significant.

These results suggest that the increased acetate suppressed the removal of the pesticides. Several plausible explanations exist for this phenomenon. The first is competitive inhibition, in which the bacteria may have preferred the acetate to the pesticide since it is easier to degrade. Schmidt and Alexander (1985) provide an example of competitive inhibition. They found that greater concentrations of acetate in an aerobic system delayed the mineralization of phenol for longer periods of time. A second possible reason for the increase in the pesticide effluent concentration is that the increased acetate resulted in greater bacterial activity, which meant the culture consumed the already limited dissolved oxygen more quickly, thus reducing the amount of oxygen available for pesticide degradation. This would be true if O_2 is part of the stoichiometry of the biotransformation of the pesticides.

During the third part of the experiment, the feeding cycle was increased to four days, doubling the contact time between the herbicides and the bacteria. The result was that the herbicide concentrations decreased to an average of 139.1 µg/l (s.d. 27.5) for propachlor and to an average of 113.2 µg/l (s.d. 26.7) for alachlor. Alachlor's effluent concentration is higher and propachlor's is lower than they were in the first period. The difference is large enough statistically to conclude that alachlor is preferred to propachlor. A couple of possible reasons exist for the reduction in the pesticide effluent concentrations. First, the increased contact time allowed for enough acetate to be

mineralized such that the effects of competitive inhibition were overcome. Second, during the four day period, the aerobic or facultative organisms present may have consumed all the oxygen within the first couple of days. Following this, facultative bacteria and/or anaerobic bacteria that subsequently became active were able to degrade the pesticides.

In the fourth and final period, the acetate was removed from the influent to test the importance of the primary substrate. The effluent concentration of the pesticides remained about the same as in the third period of the experiment. Propachlor's effluent concentration was 122.9 µg/l (s.d. 19.1) and alachlor's was 114.9 µg/l (s.d. 26.7). The difference between the two pesticides was not great enough to prove the bacteria preferred either. Since degradation continued after the acetate was removed, the suspected reasons for the continued degradation are the same as for the sterile columns, including the possible effects of dissolved organic carbon present in the inorganic salt feed solution, bacteria that are capable of utilizing the pesticides as a primary food source, and abiotic transformation of the pesticides. In addition, endogenous decay of the culture of acetate-utilizing organisms may also be supporting cometabolism of the pesticides.

One interesting result of the sterile control columns being able to degrade the pesticides is that the difference between columns being fed an added carbon source and columns that are not being fed added carbon sources can be tested. Differences between the columns was tested for with a t-test. T-tests were performed on data sets for experimental periods in which the retention times were the same. That is, the two day feeding cycle for the sterile column was tested against the two day for the aerobic columns. The differences between the columns under all of the different feeding cycles

were not statistically significant. This result is rather surprising since it indicates that the columns being fed acetate did not out perform the sterile columns. The acetate columns were expected to be able to support larger bacterial populations that could degrade the pesticides via cometabolism. This lends some credence to the thought that the degradation is accomplished either abiotically or by bacteria that are capable of using the pesticides as a food source.

Adsorption most likely played a very small role in the removal of the pesticides. Since the soil used in this project was mostly sand with very little clay or organic material and much of the very fine material was lost during column setup, the amount of adsorption is apparently very small. These issues are explored in the adsorption section further below.

Overall, these results are similar to Garrett's (1993) results in that alachlor was found to be biotransformed at a faster rate than propachlor. However, Wang (1994) and Alexander (1985) found that propachlor was biotransformed at a faster rate than alachlor.

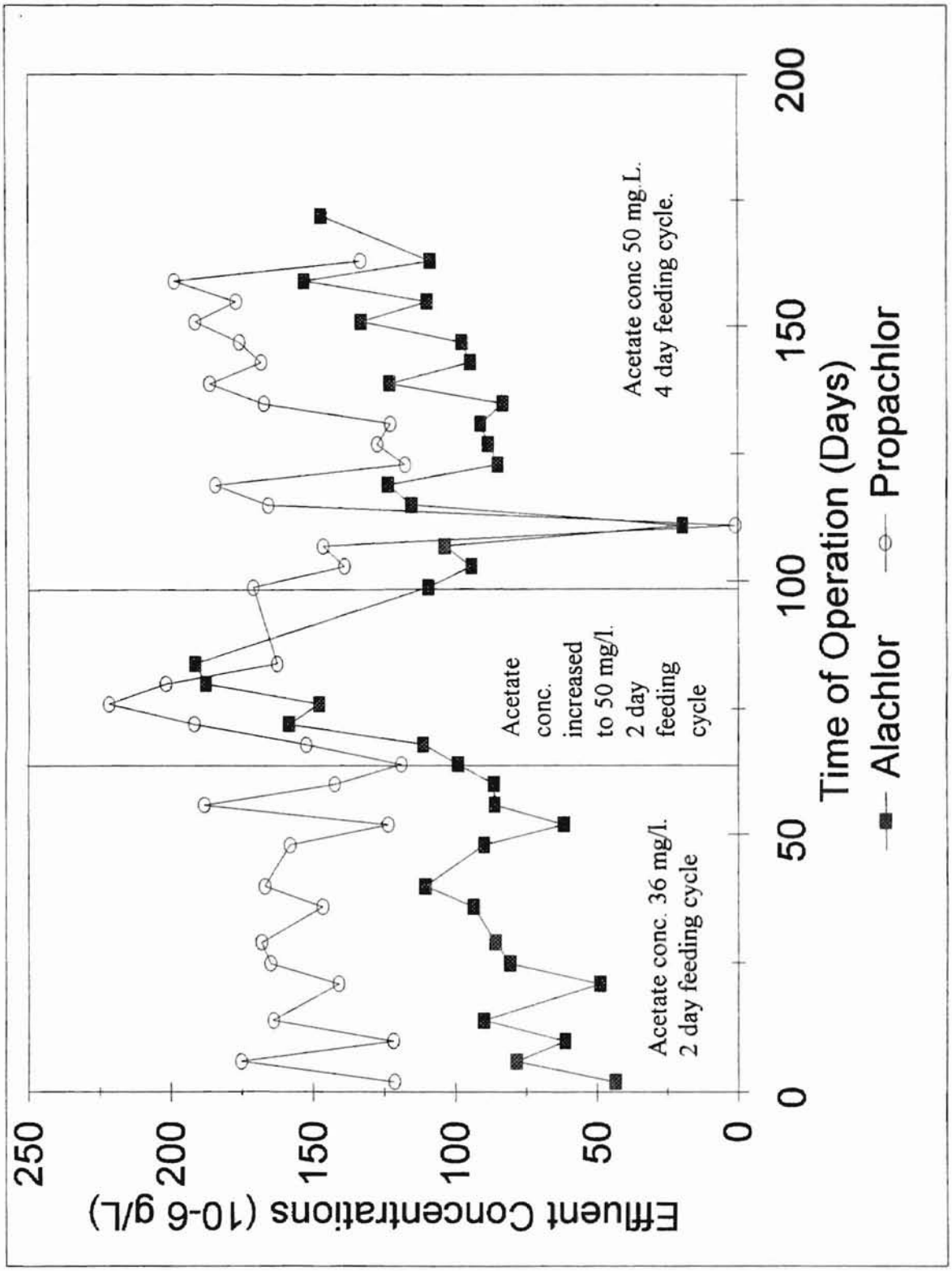


Figure 6. Aerobic Column 1

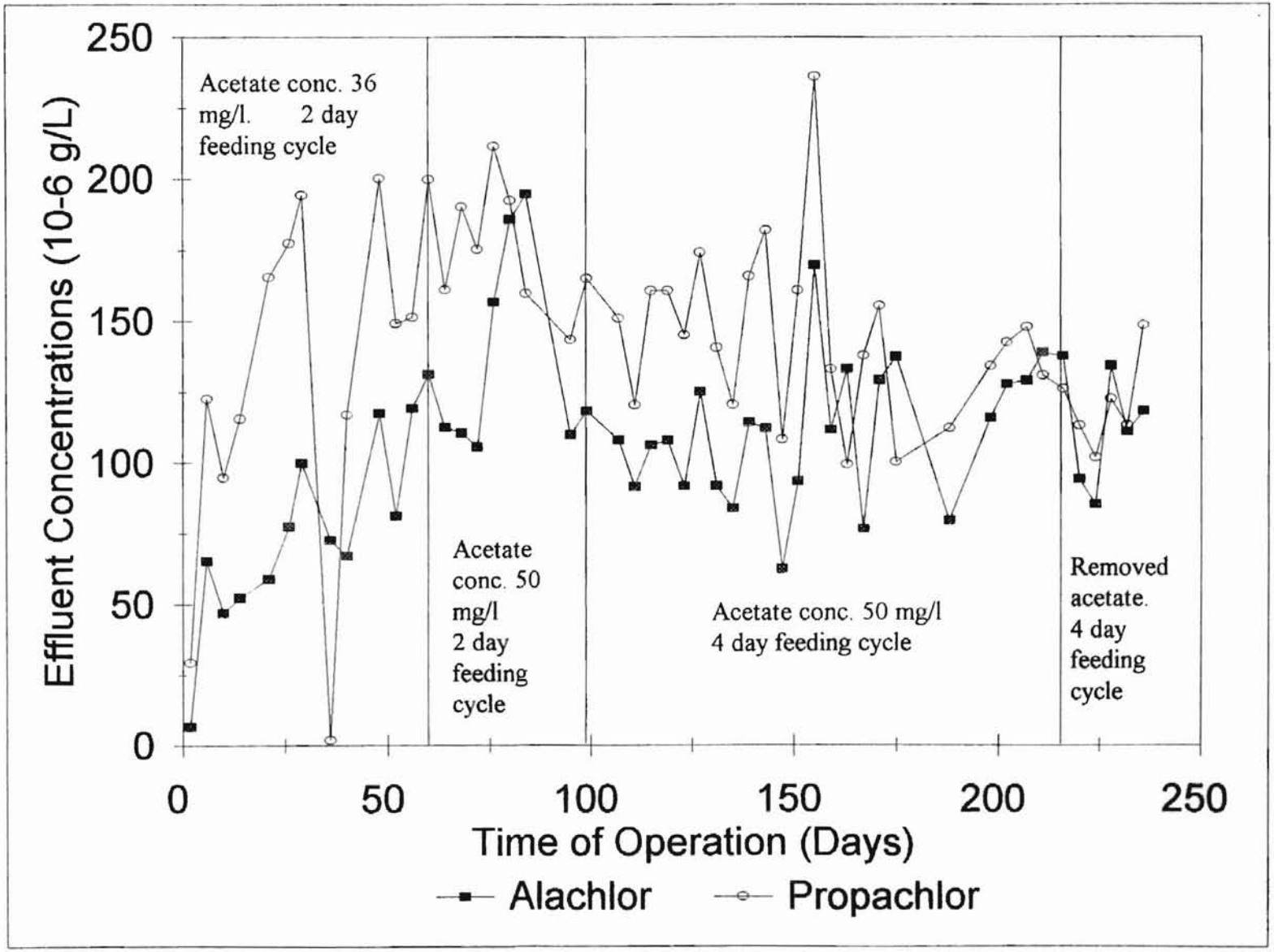


Figure 7. Aerobic Column 2

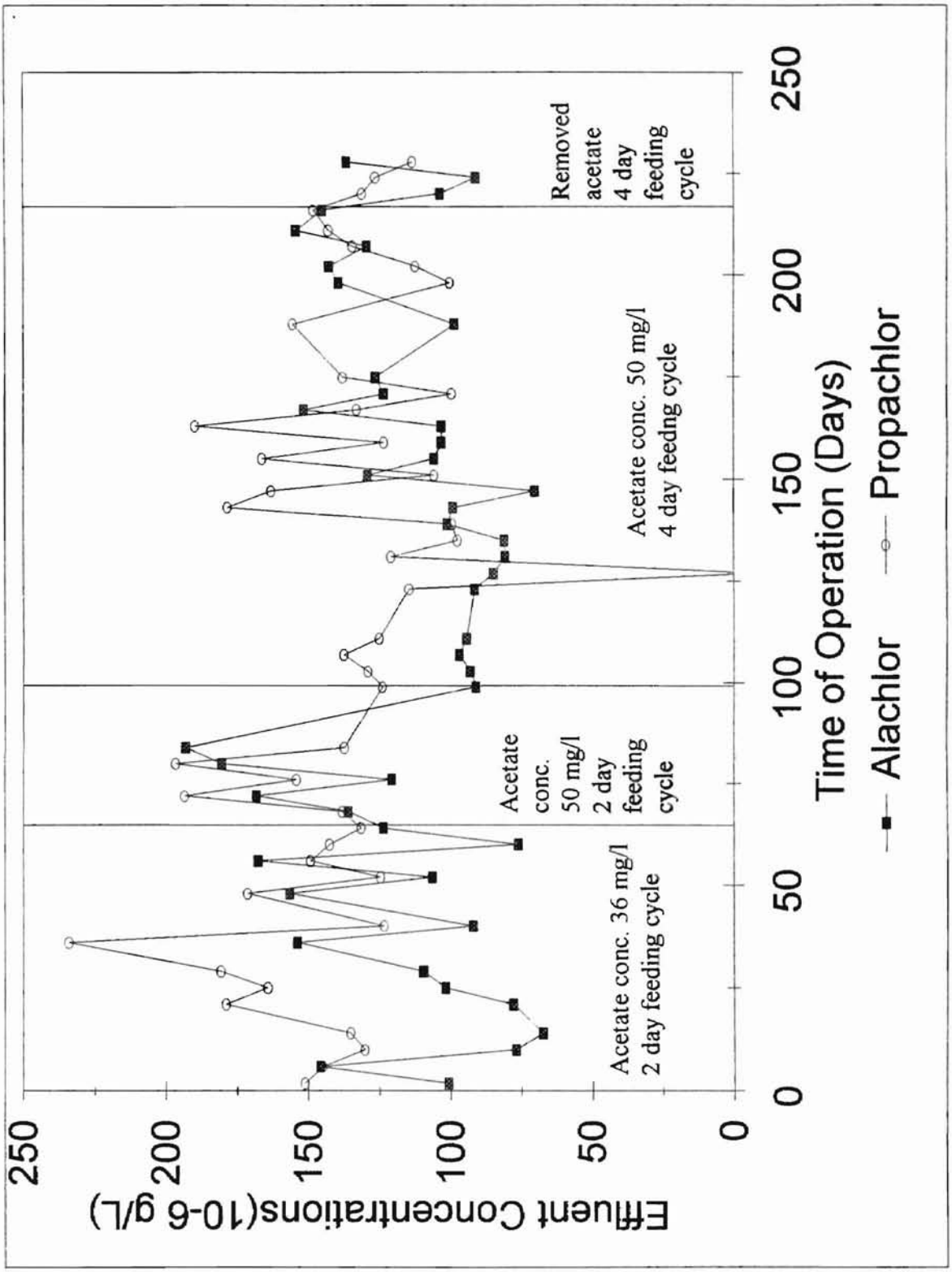


Figure 8. Aerobic Column 3

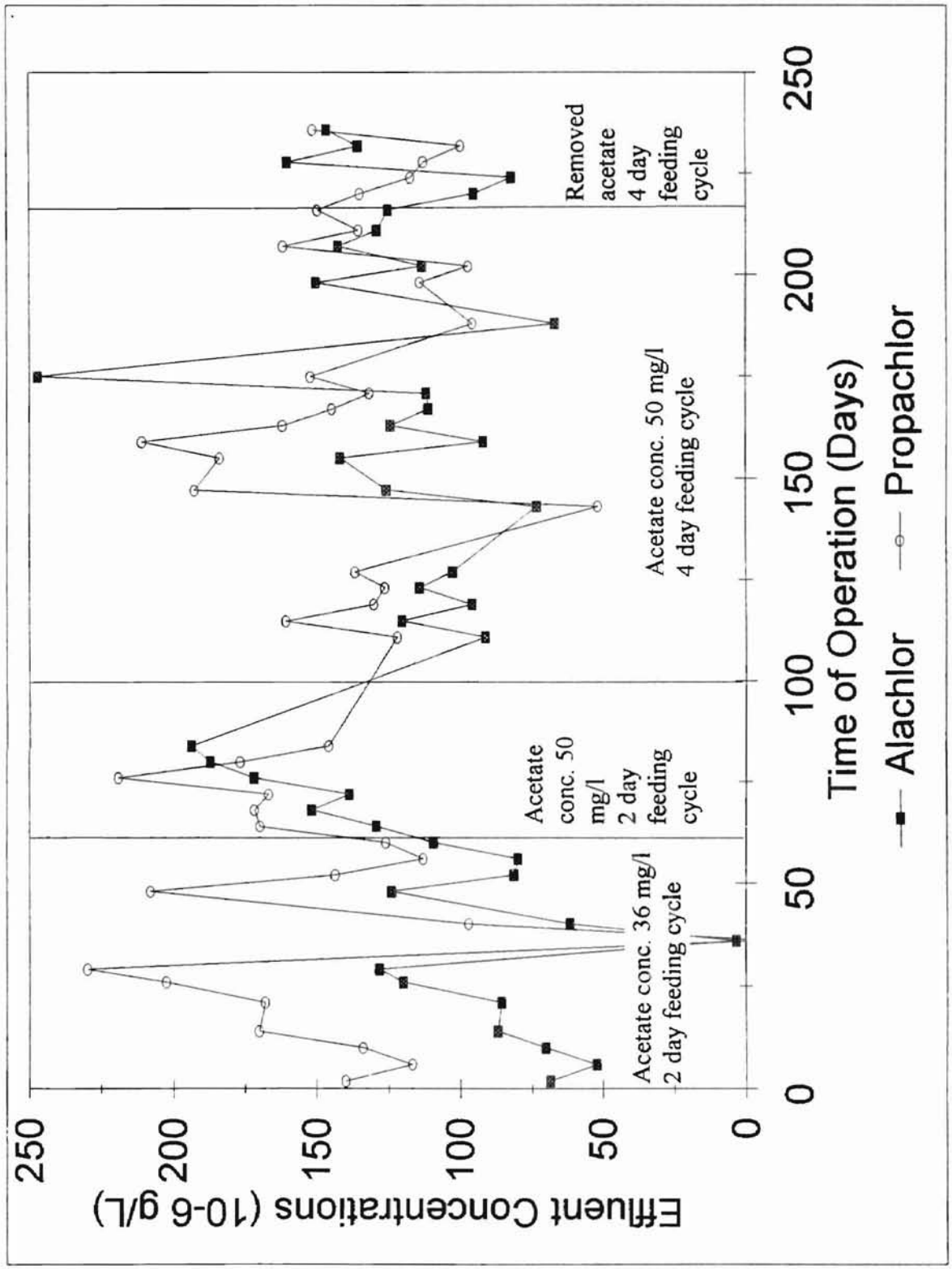


Figure 9. Aerobic Column 4

Sulfate-Reducing Columns

The purpose of these columns was to test the biodegradation of alachlor and propachlor under sulfate-reducing conditions using acetate as the primary food source. The raw data is shown in Appendixes B and C. These columns were operated under two different conditions. The first condition, operated from day 0 to 101 involved the columns being fed every four days. The influent feed was defined previously in the Methods section. The second condition, operated from day 101 to day 122, was the same as the first except that the acetate was removed from the feed.

The target acetate influent value was 80 mg/l and the average measured value was 68.5 mg/l. Acetate effluent concentrations were only measured a couple of times as an indication of microbial activity, since there were other indicators as well. These other indicators include the "rotten egg" smell from the sulfide when collecting samples and the precipitation of iron sulfides that caused the column to turn a dark gray color. Both of these indicate the presence of sulfide, which is produced under sulfate-reducing conditions. The theoretical stoichiometric usage, in a system like this, is 0.89 moles of sulfate/mole of acetate (Wang 1994). The stoichiometric equation is given below:

$$CH_3COO^- + 0.893\ SO_4^{2-} + 1.336\ H^+ + 0.043\ NH_4^+ = 0.958\ H_2O + 0.043\ C_5H_7O_2N$$
$$+ 0.829\ CO_2 + 0.446\ H_2S + 0.446\ HS^- + 0.958\ HCO_3^- \quad \ldots\ldots\ldots\ldots [5]$$

The actual usage of sulfate in sulfate-reducing columns 1 and 2 were one mole sulfate/mole acetate and 1.51 mole sulfate/mole acetate, respectively. Since these values were tested only once, these results are close enough to support the conclusion that the

columns were operating under sulfate-reducing conditions. Additional data would be needed to confirm these stoichiometric ratios.

In the beginning of the experiment, the herbicide effluent concentrations were high (see Figures 10 and 11). This is most likely due to the fact that the sulfate-reducing culture was not established prior to pesticide addition and the biomass was still growing in the first part of the experiment. Sulfate-reducing bacteria are known to grow relatively slowly, and hence, this "lag time" is not surprising.

As the bacterial population grew, the effluent concentrations dropped until there was nearly complete biotransformation of both pesticides. Once "steady-state conditions had been reached, the average concentration for alachlor and propachlor are 12.0 μg/l (s.d. 9.0) and 0 μg/l (s.d. 10.2), respectively. Alachlor's concentration is higher than propachlor's and statistically propachlor was preferred to alachlor. In comparing these results with the aerobic column results, in terms of pesticide transformation rate, it appears that sulfate respiration is clearly the more favorable condition for alachlor and propachlor biotransformation.

As mentioned previously, part of the pesticide removal under sulfate-reducing conditions is due to the reaction between bisulfide and the pesticides. Wang (1994) and Garrett (1993) reported that bisulfide reacts with alachlor and propachlor. Garrett (1993) determined a reaction rate constant of 0.0028L/h-mg [HS^-] for propachlor, and it is expressed mathematically as follows:

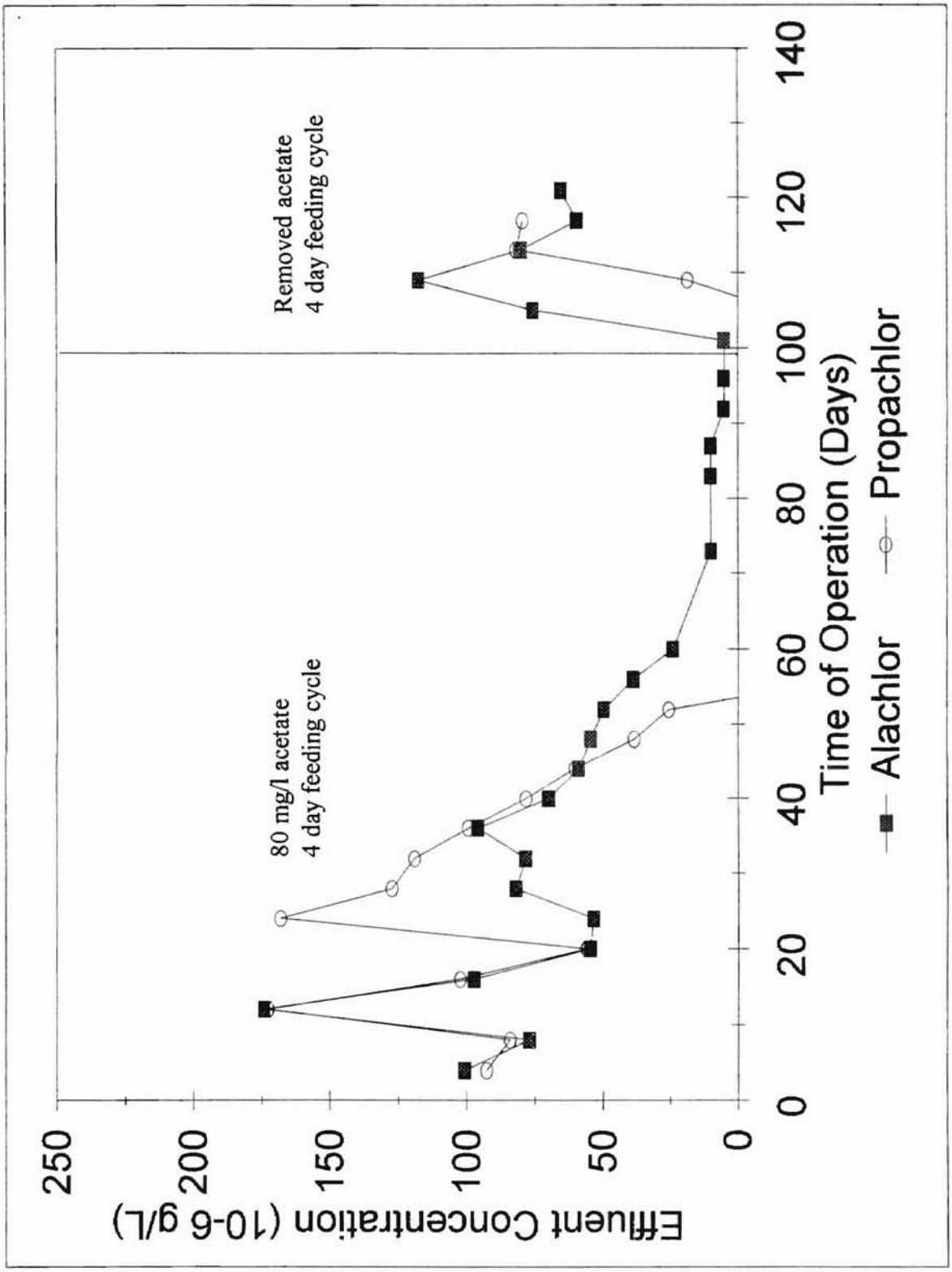


Figure 10. Sulfate Reducing Column 1

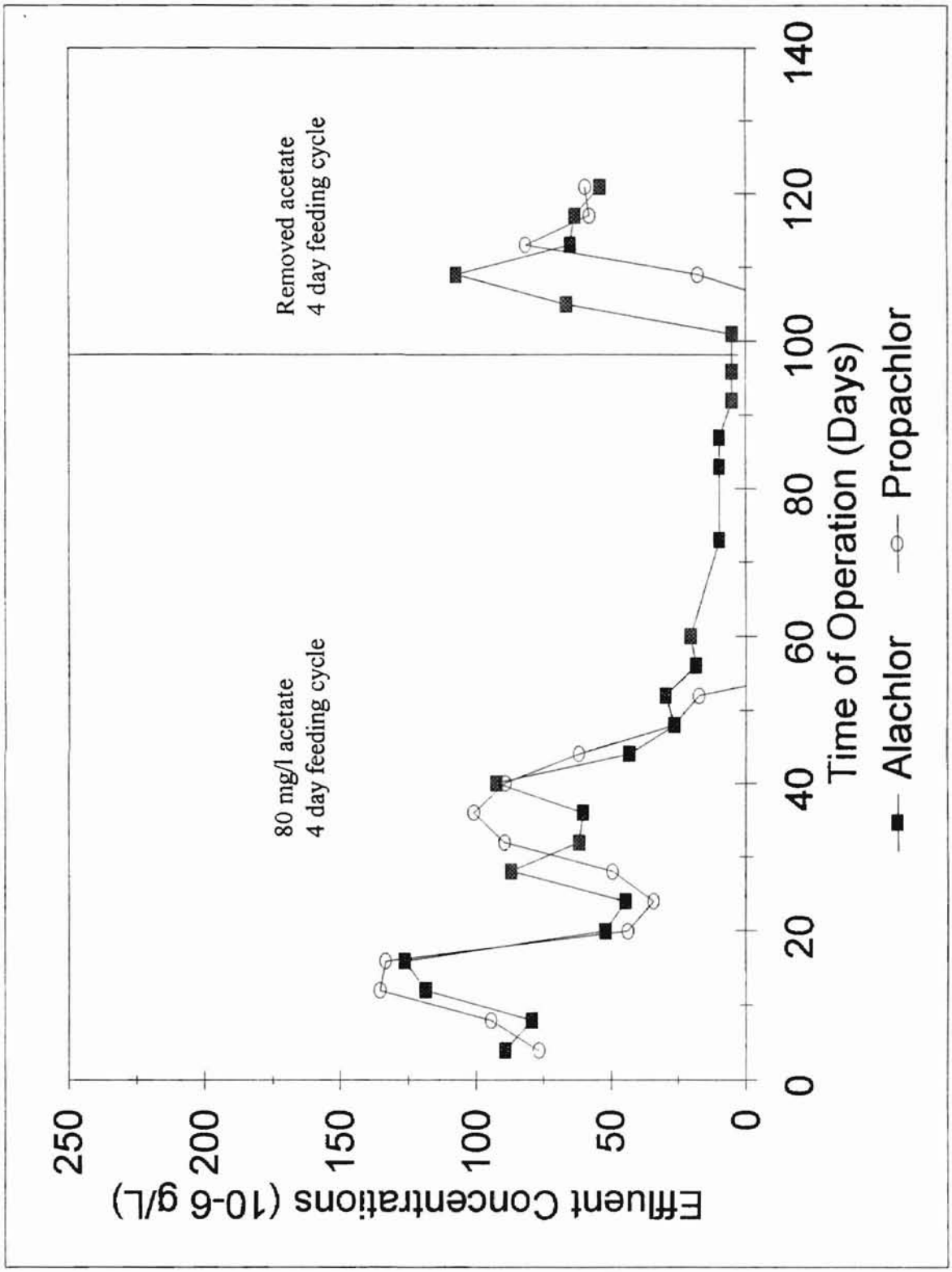


Figure 11. Sulfate Reducing Column 2

$\ln (C/Co) = 0.0028 [HS^-]t$ . . . . . [1]

C/Co = fraction remaining of pesticides

$[HS^-]$ = bisulfide concentration, mg/l

t = reaction time

Garrett (1993) also determined a reaction rate constant for alachlor of 0.0011L/h-mg $[HS^-]$, and it is expressed mathematically as follows:

$\ln (C/Co) = 0.0011 [HS^-]t$ . . . . . .[2]

In experiments with glass-bead biofilm columns with similar feed conditions to these presented here, Wang (1994) determined that with almost 100% pesticide removal, transformation by reaction with bisulfide was insignificant due to the low bisulfide concentrations in the columns. The bisulfide concentration was not measured for either of the two sulfate-reducing columns here. However, the bisulfide concentration was expected to be as low or lower than Wang's. Glass beads were used as the support matrix in Wang's columns, and the columns used in this project contained aquifer material. This material is likely to contain much more iron and other surfaces for the bisulfide to react with compared to glass beads. Hence, it can be assumed that likewise, in these columns most pesticide removal is also due to microbial activity and only partially due to abiotic reactions with bisulfide.

In the second part of the experiment, the acetate was removed from the influent. The indicators of sulfate reduction, the rotten egg odor and the dark gray color disappeared within seven to ten days. The effluent pesticide levels increased but did not

reach the influent level. After reaching an apparent steady state (see Figure 10 and 11), the average effluent levels for alachlor and propachlor were 75.3 µg/l (s.d. 21.7) and 57.1 µg/l (s.d. 27.9). Again, alachlor's concentration appears to be slightly higher than propachlor's, but a statistical difference cannot be proven. The biotransformation of the pesticides continued, but at a lower level than when acetate was present. The continuing cometabolic degradation of the herbicides could possibly be associated with endogenous decay or the bacteria using the trace amounts of organic matter in the soil or feed water as an energy source. This condition was only run for about 24 days, so it is unknown if the effluent level would eventually rise to the influent level or if it would remain less than the influent level.

These columns appeared to exhibit cometabolic pesticide degradation. When the column samples were analyzed by the GC, two extra peaks, not present in the pesticide standards, were detected. One of the peaks was after the propachlor peak and the other was before the alachlor peak. As the alachlor and propachlor peaks dwindled in size, these extra peaks increased in size until they were about the same size as the initial alachlor and propachlor peaks. These extra peaks indicated that at least two organic metabolites were produced during the biotransformation of the pesticides.

Adsorption

The ability of the pesticides to adsorb to the soil used in the research project was tested with the use of isotherm experiments. Different amounts of soil were put into flasks containing equal volumes of solution with the same concentrations of alachlor or

propachlor. After the samples had been on the shaker table for a known equilibrium time, 48 hours, the pesticide remaining in solution was extracted and tested on the GC. The results were then plotted (concentration vs. soil amount) and a linear regression performed on the data points. The slope of the linear regression line was multiplied by the aqueous volume (50 ml) used for the experiment. The R^2 value on the linear regression for both graphs is very low, 0.126 for alachlor and 0.373 for propachlor. The results are shown in Figures 12 and 13, although these low R^2 values make their meaningfulness somewhat dubious. An initial aqueous concentration of 138 μg/l was used for the alachlor experiment. At this concentration, the soils adsorbed 0.058 μg per gram of soil. The concentration used for the propachlor experiments was 231 μg/l. At this concentration, the soil adsorbed 0.39 μg per gram of soil. Even with there being a slight difference between the initial concentrations of alachlor and propachlor, the difference between the adsorption values is great enough to conclude that propachlor is considerably adsorbed more than alachlor by the aquifer material.

Freundlich isotherms were also attempted with this data set. Due to the wide variability of the data, the Freundlich isotherms were useless. The results appear in Appendix F.

The purpose of the sterile columns was also to describe the effect of adsorption and its effect on bioavailability. Several problems were encountered in this effort. The first problem, discussed earlier, was the degradation of the pesticides through either biotic or abiotic reactions. Another set of problems was encountered when an effort was made to desorb the pesticides from the soil. The first method used to desorb the pesticide from

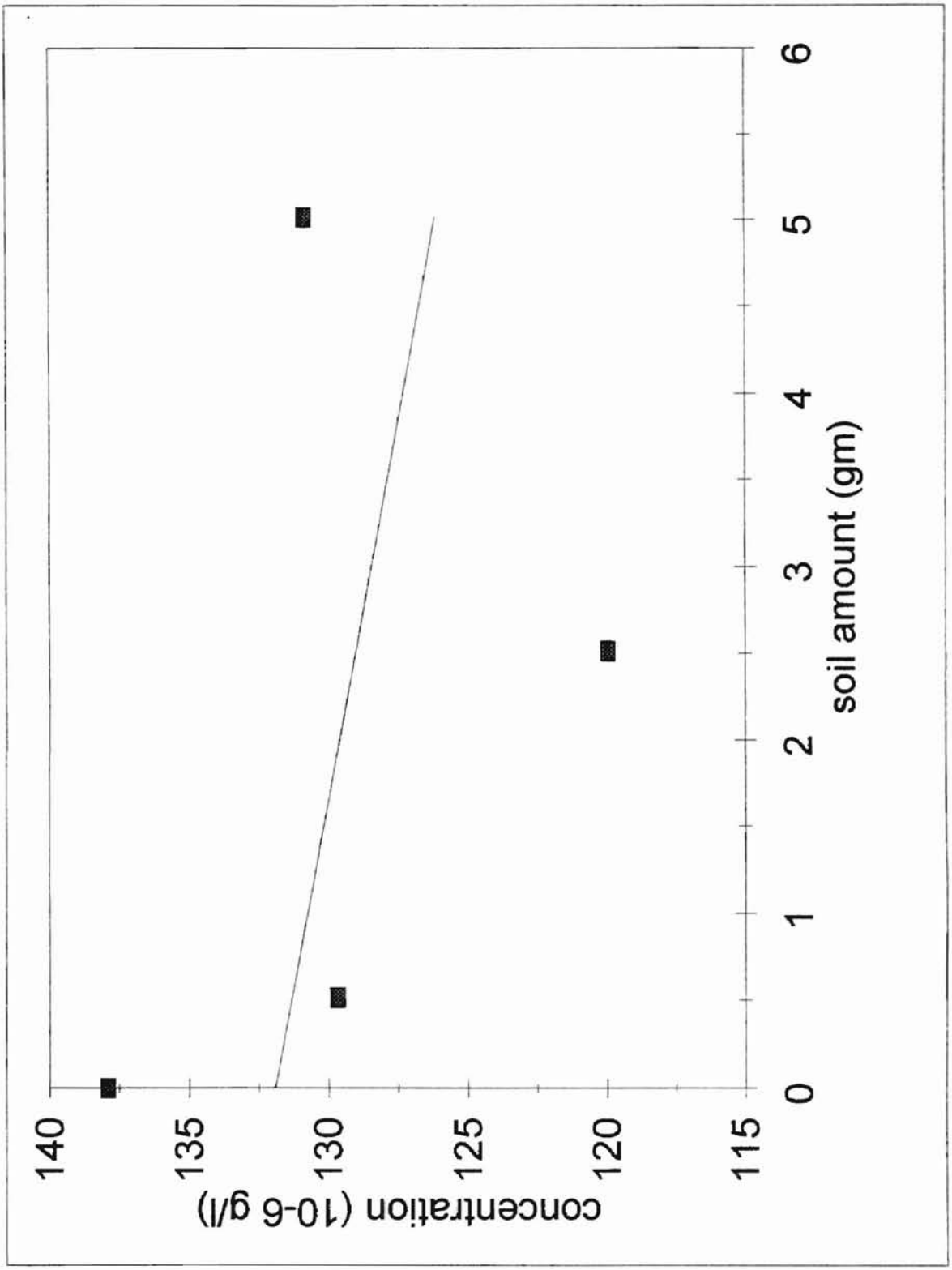


Figure 12. Alachlor Concentrations in Varying Amounts of Soil

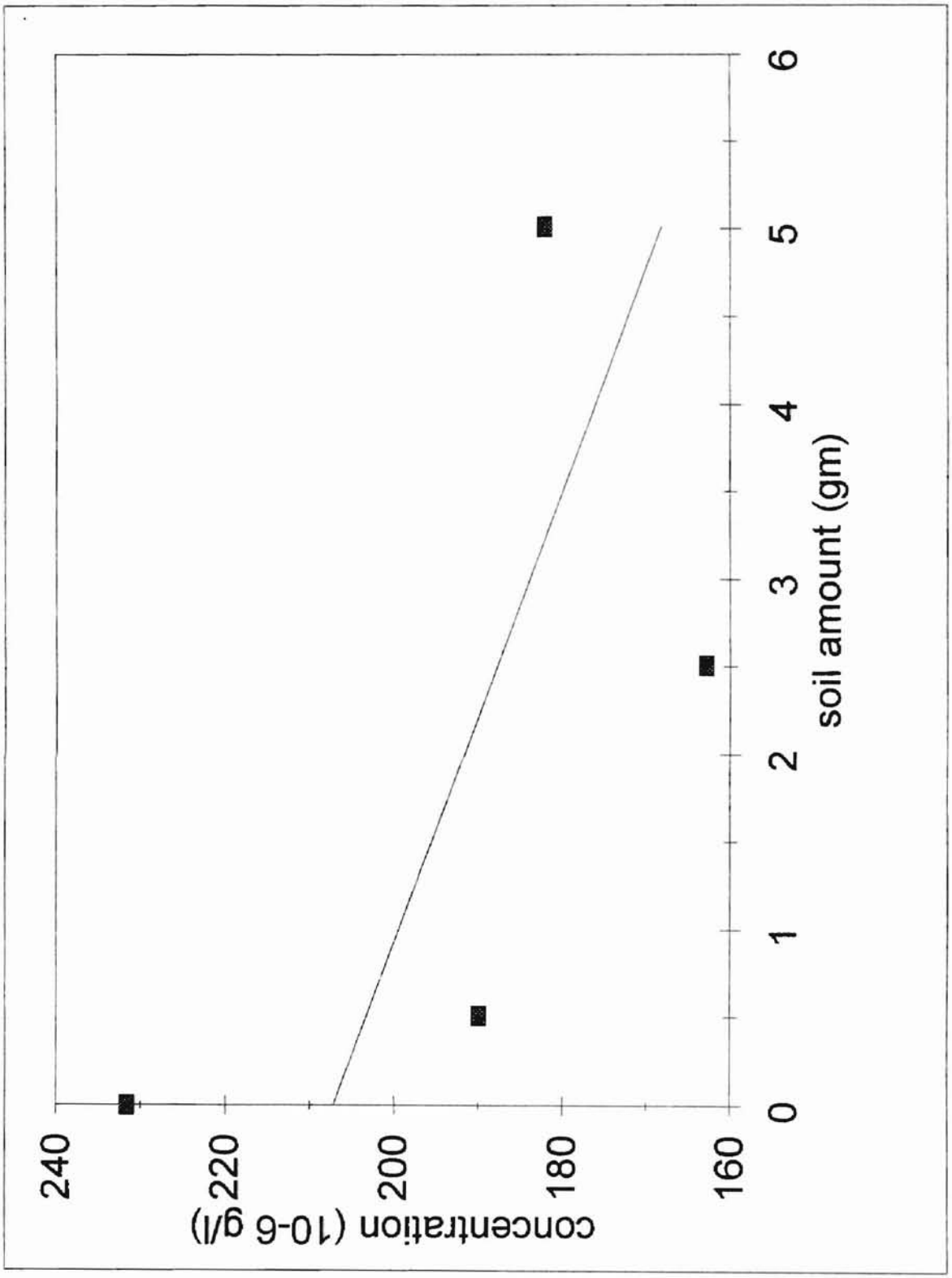


Figure 13. Propachlor Concentrations in Varying Amounts of Soil

the soil was that described by Huang and Pignatello (1990). This method, uses methanol as the extractant, was selected since in previous work it had exhibited good extraction efficiencies with few background peaks. However, during the current study this was not the case. Using this method, the expected concentrations, based on the above isotherm experiments, for alachlor and propachlor are 2.3 and 15.7 µg/l, respectively. When the samples were ran on the GC, the detector was overwhelmed with noise and the noise level did not decrease until after the time required for the pesticides to come off the column. This extraction was attempted several times with similar poor results. Because of the background noise, there were not any usable results from running the samples on the GC.

The second method used to desorb the pesticide from the soil was described by Guo et al. (1993). Using ethyl acetate as the extractant, this method exhibited a 74% recovery rate for alachlor. The expected concentrations, in the extracted liquid volumes prescribed by the method, for alachlor and propachlor are 3 and 20 µg/l, respectively. While the background signal noise encountered with these samples was low enough to permit detection of the pesticides, the GC did not detect any pesticide in these samples. There are a couple of possible explanations. First, it is possible that none of the adsorbed pesticide was desorbed from the soil. This seems less likely since the solvent (ethyl acetate) used readily desorb the pesticides from the C_{18} cartridge that is used in the aqueous analysis. Second, and more likely, the amount of pesticide desorbed from the soil was too low to be detected by the GC This is the more likely explanation since the amount of pesticide adsorbed by the soil during the isotherm experiments was also very small.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Using the pesticides alachlor and propachlor, this research project attempted to answer questions about soil adsorption, the effects of adding an external carbon source, and the effects of electron acceptor conditions on biotransformation. The specific objectives this study tried to answer are listed below:

1) Evaluate the biotransformation of the herbicides alachlor and propachlor in soil-water systems under aerobic and sulfate-reducing conditions.
2) Describe the effect of sorption on these systems and evaluate its impact on the availability of pesticides for biotransformation.
3) Further investigate the effect of acetate as an added carbon source on such systems.

This project was unable to meet the goal of definitively answering questions about adsorption because the mass balance on the pesticides could not be closed. The mass balance could not be closed for a couple of reasons. First, the pesticides in the sterile columns were degraded through either biotic or abiotic processes, making them useless as controls. Secondly, the amount of pesticide adsorbed could not be quantified, due to problems with the desorption experiments, including background noise on the GC, or

adsorption below the detection limit of the methods.

The main findings of this study are the following:

1) Sulfate respiration is more favorable than aerobic respiration (in terms of removal rate) for the biotransformation of propachlor and alachlor.
2) Loss of the primary substrate had a significant effect on the sulfate reducing columns.
3) Sorption did not appear to be a significant removal mechanism for either alachlor or propachlor.
4) In aerobic systems, higher concentrations of acetate inhibited the degradation of alachlor and propachlor.
5) Without a primary food source, the sterile columns were able to degrade alachlor and propachlor.
6) No significant difference was observed in the pesticide transformation ability between columns receiving an added carbon source and columns that were not.
7) Organisms appeared to be resistant to sterilization techniques that were used.

Recommendations

There are several areas that this project can be improved. The sterile columns need to be completely sterilized and then kept sterile. According to Wolf et al. (1989), autoclaving the soil once for two hours will not kill all the bacteria. The soil needs to be autoclaved at least twice. To keep the columns sterile, the feed can be autoclaved before feeding the columns or sodium azide can be added to the feed to keep biological growth to

a minimum. Another area that needs to be addressed is pesticide desorption. First, enough pesticide needs to be adsorbed so that when it is desorbed the concentration is great enough to be measured. This can be accomplished by using a large volume of soils or soils that contain high amounts of organic matter. A reliable method of desorbing pesticides, with high recovery rates, needs to be found. One possible method is the use of radio-labeled pesticides.

Some areas of further research can also be suggested. Since the amount of degradation decreased with an increase in the acetate concentration, it should be determined what is the optimum amount of acetate feed. Also the abiotic degradation of pesticides in aquifers warrants further investigation, including determining the rate and mechanism of these reactions.

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APPENDIXES

APPENDIX A
BROMIDE TRACER STUDY

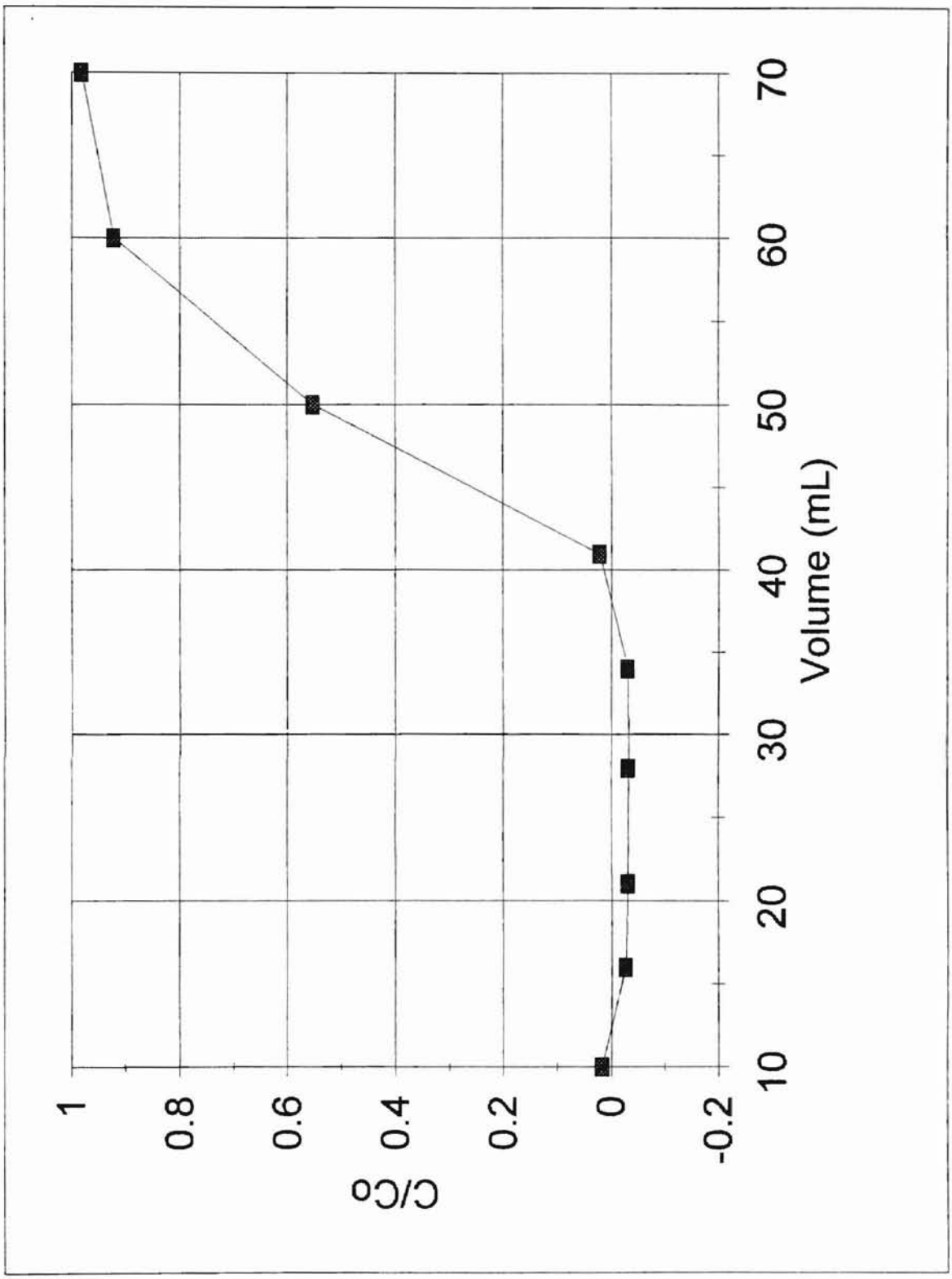
Bromide tracer column 1

vol.	Area	Conc.	C/Co
10	127576	1.7841696	0.0178417
16	14692	-2.740124	-0.027401
21	5682	-3.101237	-0.031012
28	2787	-3.217266	-0.032173
34	4460	-3.150213	-0.031502
41	136076	2.1248423	0.0212484
50	1460388	55.202124	0.5520212
60	2382907	92.17589	0.9217589
70	2533649	98.217499	0.982175

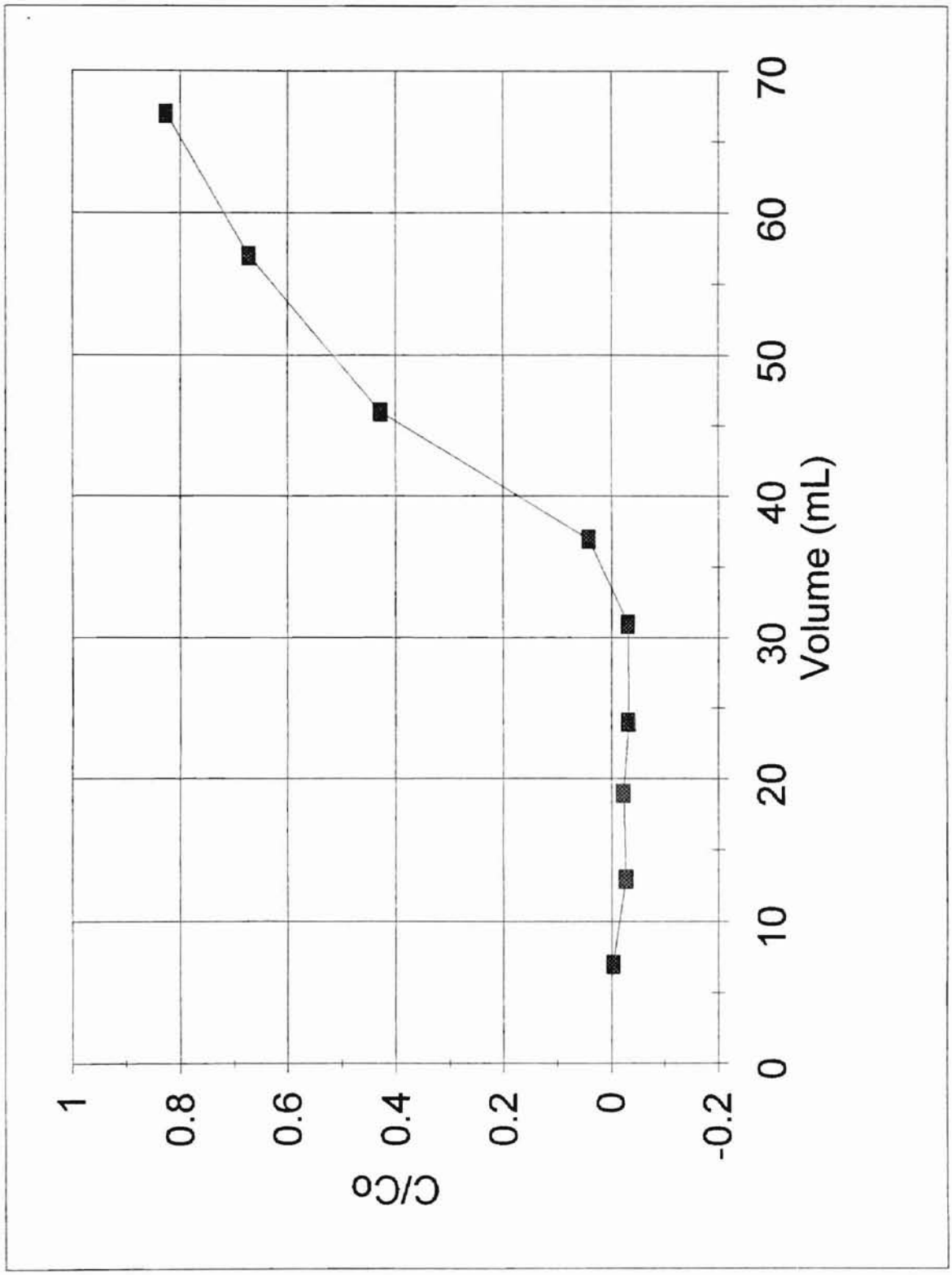
Bromide tracer column 2

Vol.	Area	Conc.	C/Co
7	74315	-0.350485	-0.003505
13	14924	-2.730825	-0.027308
19	27104	-2.242661	-0.022427
24	3196	-3.200873	-0.032009
31	3655	-3.182477	-0.031825
37	188539	4.227514	0.0422751
46	148496	42.701762	0.4270176
57	1760307	67.222619	0.6722262
67	2144874	82.635732	0.8263573

initial Br- conc. was 100 mg/L



Bromide Tracer Study Column 1



Bromide Tracer Study Column 2

APPENDIX B

RAW DATA, PROPACHLOR EFFLUENT CONCENTRATIONS

Aerobic Column 1

Date	Conc.(µg/l)	Days	Date	Conc..(µg/l)	Days
02/03/95	121.59	2	04/26/95	162.69	84
02/07/95	175.40	6	05/11/95	171.03	99
02/11/95	121.90	10	05/15/95	138.95	103
02/15/95	164.09	14	05/19/95	146.56	107
02/22/95	141.12	21	05/23/95	0.88	111
02/27/95	165.04	25	05/27/95	165.69	115
03/02/95	168.22	29	05/31/95	184.31	119
03/09/95	146.87	36	06/04/95	117.57	123
03/13/95	167.03	40	06/08/95	127.46	127
03/21/95	158.35	48	06/12/95	122.75	131
03/25/95	123.75	52	06/16/95	167.27	135
03/29/95	188.25	56	06/20/95	186.26	139
04/02/95	142.45	60	06/24/95	168.24	143
04/06/95	119.11	64	06/28/95	175.89	147
04/10/95	152.59	68	07/02/95	191.47	151
04/14/95	191.98	72	07/06/95	177.10	155
04/18/95	222.08	76	07/10/95	198.85	159
04/22/95	202.01	80	07/14/95	133.15	163

Aerobic Column 2

Date	Conc.(µg/l)	Days	Date	Conc.(µg/l)	Days
02/03/95	29.56	2	06/04/95	145.13	123
02/07/95	122.83	6	06/08/95	174.11	127
02/11/95	94.88	10	06/12/95	140.76	131
02/15/95	115.80	14	06/16/95	120.52	135
02/22/95	165.51	21	06/20/95	165.94	139
02/27/95	177.58	26	06/24/95	181.99	143
03/02/95	194.47	29	06/28/95	108.27	147
03/09/95	1.96	36	07/02/95	160.92	151
03/13/95	117.11	40	07/06/95	236.26	155
03/21/95	200.35	48	07/14/95	133.02	163
03/25/95	149.37	52	07/18/95	99.46	167
03/29/95	151.47	56	07/22/95	137.83	171
04/02/95	199.93	60	07/26/95	155.40	175
04/06/95	161.14	64	08/03/95	100.23	183
04/10/95	190.29	68	08/08/95	112.26	188
04/14/95	175.43	72	08/18/95	134.43	198
04/18/95	211.64	76	08/22/95	142.76	202
04/22/95	192.74	80	08/27/95	148.11	207
04/26/95	159.85	84	08/31/95	130.85	211
05/11/95	143.43	99	09/05/95	126.24	216
05/15/95	165.08	103	09/09/95	113.28	220
05/19/95	151.09	107	09/13/95	101.98	224
05/23/95	120.45	111	09/17/95	122.51	228
05/27/95	160.77	115	09/21/95	113.31	232
05/31/95	160.73	119	09/25/95	148.60	236

Aerobic Column 3

Date	Conc.(µg/l)	Days	Date	Conc.(µg/l)	Days
02/03/95	151.28	2	06/08/95	120.93332	127
02/07/95	145.07	6	06/12/95	97.553078	131
02/11/95	130.25	10	06/16/95	99.58125	135
02/15/95	135.27	21	06/24/95	163.1	143
02/26/95	164.3	26	6*/28/95	105.69004	147
03/02/95	180.65011	29	7*/2/95	166.18859	151
03/09/95	234.08798	36	7*/6/95	123.38086	155
03/13/95	123.34978	40	07/10/95	189.87148	159
03/21/95	171.45463	48	07/14/95	133.02089	163
03/25/95	124.83023	52	07/18/95	99.457559	167
03/29/95	149.16334	56	07/22/95	137.83428	171
04/02/95	142.51662	60	07/26/95	155.39625	175
04/06/95	131.61299	64	08/03/95	100.23009	183
04/10/95	138.13231	68	08/08/95	112.25976	188
04/14/95	193.46199	72	08/18/95	134.43327	198
04/18/95	154.18248	76	08/22/95	142.76488	202
04/22/95	196.66413	80	08/27/95	148.10795	207
04/26/95	137.43298	84	08/31/95	130.85336	211
05/11/95	123.87241	99	09/05/95	126.2391	216
05/15/95	129.14678	103	09/09/95	113.27529	220
05/19/95	137.33846	107	09/13/95	101.9784	224
05/23/95	125.01642	111	09/17/95	122.50937	228
05/27/95	114.54492	115	09/21/95	113.30762	232
05/31/95	-8.208161	123	09/25/95	148.59588	236

Aerobic Column 4

Date	Conc.(µg/l)	Days	Date	Conc.(µg/l)	Days
02/03/95	139.94	2	06/08/95	136.60	127
02/07/95	116.87	6	06/12/95	51.88	143
02/11/95	134.02	10	6/28/95	192.57	147
02/15/95	170.11	14	7/2/95	183.89	151
02/22/95	168.01	21	7/6/95	211.05	155
02/26/95	202.56	26	07/10/95	161.97	159
03/02/95	229.87	29	07/14/95	144.64	163
03/09/95	-7.29	36	07/18/95	131.55	167
03/13/95	97.25	40	07/22/95	152.23	171
03/21/95	208.09	48	07/26/95	95.61	175
03/25/95	143.67	52	08/03/95	114.01	183
03/29/95	113.13	56	08/08/95	97.07	188
04/02/95	126.14	60	08/18/95	161.83	198
04/06/95	169.74	64	08/22/95	135.30	202
04/10/95	171.82	68	08/27/95	149.57	207
04/14/95	166.80	72	08/31/95	134.81	211
04/18/95	219.10	76	09/05/95	117.09	216
04/22/95	176.71	80	09/09/95	112.66	220
04/26/95	145.94	84	09/13/95	99.79	224
05/15/95	121.92	103	09/17/95	151.34	228
05/19/95	160.70	107	09/21/95	137.07	232
05/23/95	130.03	119	09/25/95	143.09	236
06/04/95	126.27	123			

Sterile Column 1

Date	Conc.(µg/l)	Days	Date	Conc.(µg/l)	Days
			06/12/95	141.04	106
03/09/95	182.50	11	06/16/95	107.85	110
03/13/95	119.14	15	06/20/95	138.80	114
03/21/95	90.60	23	06/28/95	120.79	122
03/25/95	125.99	27	07/02/95	154.71	126
03/29/95	170.19	31	07/06/95	153.40	130
04/02/95	202.54	35	07/10/95	208.29	134
04/06/95	146.20	39	07/18/95	141.68	142
04/10/95	147.92	43	07/22/95	112.00	146
04/14/95	241.33	47	07/26/95	110.56	150
04/18/95	123.86	51	08/03/95	86.11	158
04/26/95	171.95	59	08/08/95	81.34	163
05/11/95	241.90	74	08/22/95	169.02	177
05/15/95	190.23	78	08/27/95	164.29	182
05/19/95	178.70	82	08/31/95	145.78	186
05/27/95	163.85	90	09/05/95	173.23	191
05/31/95	220.86	94	09/09/95	149.70	195
06/08/95	214.75	102	09/13/95	144.26	199

Sterile Column 2

Date	Conc.(µg/l)	Days	Date	Conc.(µg/l)	Days
03/09/95	67.99	11	06/28/95	82.01	122
03/13/95	63.53	15	07/02/95	103.63	126
03/21/95	86.52	23	07/06/95	103.74	130
03/25/95	121.38	27	07/10/95	136.20	134
03/29/95	158.41	31	07/14/95	107.09	138
04/02/95	128.42	35	07/18/95	111.19	142
04/06/95	138.53	39	07/22/95	32.77	146
04/10/95	256.16	43	07/26/95	129.46	150
04/14/95	130.88	47	08/03/95	62.39	158
04/18/95	203.86	51	08/08/95	78.90	163
04/22/95	107.12	55	08/18/95	153.80	173
04/26/95	252.46	59	08/22/95	149.23	177
05/11/95	200.16	74	08/27/95	134.55	182
05/15/95	149.05	78	08/31/95	135.11	186
05/19/95	146.19	82	09/09/95	117.46	195
05/23/95	152.10	86	09/13/95	103.33	199
05/27/95	191.69	90	09/17/95	131.21	203
05/31/95	185.12	94	09/21/95	163.77	207
06/04/95	137.99	98	09/25/95	158.28	211
06/08/95	174.94	102	09/29/95	169.90	215
06/12/95	117.46	106	10/08/95	217.36	224
06/16/95	86.69	110	10/12/95	29.55	228
06/20/95	124.52	114			

Sulfate Column 1

Date	Conc.(µg/l)	Days	Date	Conc.(µg/l)	Days
05/31/95	92.76	4	07/22/95	-36.02	56
06/04/95	84.04	8	07/26/95	-36.02	66
06/08/95	172.55	12	08/03/95	-24.44	70
06/12/95	102.30	16	08/08/95	-10.80	75
06/16/95	55.83	20	08/18/95	-10.80	85
06/20/95	167.91	24	08/22/95	-10.80	89
06/24/95	127.26	28	08/27/95	-16.07	93
06/28/95	119.09	32	08/31/95	-16.07	97
07/02/95	99.26	36	09/05/95	-16.07	102
07/06/95	78.19	40	09/09/95	18.61	106
07/10/95	60.21	44	09/13/95	81.60	110
07/14/95	38.51	48	09/25/95	79.03	122
07/18/95	25.67	52			

Sulfate Column 2

Date	Conc.(µg/l)	Days	Date	Conc.(µg/l)	Days
05/31/95	76.88	4	07/26/95	-36.02	60
06/04/95	94.59	8	08/03/95	-24.44	70
06/08/95	135.61	12	08/08/95	-10.80	75
06/12/95	133.42	16	08/18/95	-10.80	85
06/16/95	43.92	20	08/22/95	-10.80	89
06/20/95	34.33	24	08/27/95	-16.07	93
06/24/95	49.70	28	08/31/95	-16.07	97
06/28/95	89.49	32	09/05/95	-16.07	102
07/02/95	100.84	36	09/09/95	17.90	106
07/06/95	89.15	40	09/13/95	81.45	110
07/10/95	62.08	44	09/17/95	57.84	114
07/14/95	26.87	48	09/21/95	59.47	118
07/18/95	17.56	52	09/25/95	60.53	122
07/22/95	-36.02	56			

APPENDIX C

RAW DATA, ALACHLOR EFFLUENT CONCENTRATIONS

Aerobic Column 1

Date	Conc.(µg/l)	Days	Date	Conc.(µg/l)	Days
02/03/95	43.52	2	05/11/95	109.51	99
02/07/95	78.64	6	05/15/95	94.25	103
02/11/95	61.30	10	05/19/95	103.72	107
02/15/95	90.08	14	05/23/95	19.69	111
02/22/95	49.00	21	05/27/95	115.31	115
02/26/95	80.80	25	05/31/95	123.58	119
03/02/95	85.97	29	06/04/95	84.74	123
03/09/95	93.75	36	06/08/95	88.01	127
03/13/95	110.72	40	06/12/95	90.86	131
03/21/95	89.91	48	06/16/95	82.99	135
03/25/95	61.84	52	06/20/95	122.90	139
03/29/95	86.24	56	06/24/95	94.39	143
04/02/95	86.33	60	06/28/95	97.41	147
04/06/95	99.00	64	07/02/95	133.08	151
04/10/95	111.39	68	07/06/95	109.58	155
04/14/95	158.56	72	07/10/95	153.04	159
04/18/95	148.06	76	07/14/95	108.43	163
04/22/95	187.88	80	07/22/95	146.95	172
04/26/95	191.63	84			

Aerobic Column 2

Date	Conc.(µg/l)	Days	Date	Conc.(µg/l)	Days
02/03/95	6.72	2	06/04/95	91.74	123
02/07/95	65.37	6	06/08/95	125.04	127
02/11/95	46.99	10	06/12/95	91.74	131
02/15/95	52.45	14	06/16/95	83.75	135
02/22/95	59.12	21	06/20/95	114.44	139
02/26/95	77.60	26	06/24/95	112.02	143
03/02/95	100.16	29	06/28/95	62.51	147
03/09/95	73.01	36	07/02/95	93.48	151
03/13/95	67.37	40	07/06/95	169.72	155
03/21/95	117.54	48	07/10/95	111.62	159
03/25/95	81.52	52	07/14/95	133.05	163
03/29/95	119.37	56	07/18/95	76.62	167
04/02/95	131.42	60	07/22/95	129.07	171
04/06/95	112.67	64	07/26/95	137.38	175
04/10/95	110.69	68	08/08/95	79.65	188
04/14/95	105.73	72	08/18/95	115.88	198
04/18/95	156.63	76	08/22/95	127.92	202
04/22/95	185.86	80	08/27/95	129.15	207
04/26/95	194.75	84	08/31/95	138.85	211
05/11/95	109.89	95	09/05/95	137.65	216
05/15/95	118.33	99	09/09/95	94.27	220
05/19/95	108.10	107	09/13/95	85.31	224
05/23/95	91.65	111	09/17/95	134.20	228
05/27/95	106.19	115	09/21/95	111.15	232
05/31/95	108.12	119	09/25/95	118.22	236

Aerobic Column 3

Date	Conc.(µg/l)	Days	Date	Conc.(µg/l)	Days
02/03/95	100.74	2	05/27/95	91.58	123
02/07/95	145.72	6	06/08/95	84.72	127
02/11/95	76.88	10	06/12/95	80.56	131
02/15/95	67.42	14	06/16/95	80.89	135
02/22/95	77.90	21	06/20/95	100.99	139
02/26/95	101.88	25	06/24/95	99.05	143
03/02/95	109.49	29	06/28/95	70.07	147
03/09/95	153.83	36	07/02/95	129.03	151
03/13/95	91.98	40	07/06/95	105.70	155
03/21/95	156.68	48	07/10/95	103.25	159
03/25/95	106.53	52	07/14/95	103.14	163
03/29/95	167.62	56	07/18/95	151.56	167
04/02/95	76.14	60	07/22/95	123.39	171
04/06/95	123.58	64	07/26/95	126.37	175
04/10/95	135.80	68	08/08/95	98.60	188
04/14/95	168.19	72	08/18/95	139.18	198
04/18/95	120.60	76	08/22/95	142.72	202
04/22/95	180.29	80	08/27/95	129.31	207
04/26/95	193.13	84	08/31/95	154.16	211
05/11/95	90.96	99	09/05/95	145.08	216
05/15/95	93.01	103	09/09/95	103.46	220
05/19/95	96.86	107	09/13/95	90.97	224
05/23/95	94.28	111	09/17/95	136.40	228

Aerobic Column 4

Date	Conc.(µg/l)	Days	Date	Conc.(µg/l)	Days
02/03/95	68.75	2	05/27/95	120.19	115
02/07/95	52.48	6	06/08/95	102.73	127
02/11/95	70.27	10	06/12/95	73.19	143
02/15/95	87.10	14	06/28/95	125.61	147
02/22/95	85.70	21	07/02/95	141.79	155
02/26/95	119.88	26	07/10/95	91.77	159
03/02/95	128.31	29	07/14/95	124.29	163
03/09/95	3.31	36	07/18/95	111.02	167
03/13/95	61.92	40	07/22/95	111.74	171
03/21/95	123.94	48	07/26/95	247.08	175
03/25/95	81.47	52	08/08/95	66.88	188
03/29/95	80.13	56	08/18/95	150.16	198
04/02/95	109.55	60	08/22/95	113.10	202
04/06/95	129.33	64	08/27/95	142.60	207
04/10/95	151.81	68	08/31/95	128.78	211
04/14/95	138.65	72	09/05/95	125.05	216
04/18/95	171.72	76	09/09/95	95.19	220
04/22/95	187.03	80	09/13/95	81.93	224
04/26/95	193.79	84	09/17/95	160.16	228
05/23/95	91.04	111	09/21/95	135.54	232
05/31/95	95.85	119	09/25/95	146.34	236
06/04/95	113.97	123			

Sterile Column 1

Date	Conc.(µg/l)	Days	Date	Conc.(µg/l)	Days
03/02/95	150.10	4	06/12/95	69.91	106
03/09/95	55.78	11	06/16/95	47.45	110
03/13/95	33.28	15	06/20/95	22.61	114
03/21/95	74.45	23	06/28/95	105.21	122
03/25/95	104.47	27	07/02/95	89.44	126
03/29/95	67.18	31	07/06/95	68.52	130
04/02/95	50.43	35	07/10/95	95.39	134
04/06/95	60.64	39	07/14/95	66.80	138
04/10/95	110.29	43	07/18/95	51.95	142
04/14/95	66.49	47	07/22/95	57.42	146
04/18/95	146.47	51	07/26/95	71.86	150
04/26/95	187.41	59	08/18/95	141.44	173
05/11/95	105.27	74	08/22/95	125.50	177
05/15/95	112.98	78	08/27/95	109.87	182
05/19/95	125.63	82	08/31/95	122.75	186
05/27/95	128.79	90	09/05/95	120.52	191
05/31/95	150.49	94	09/09/95	110.05	195
06/08/95	129.23	102			

Sterile Column 2

Date	Conc.(µg/l)	Days	Date	Conc.(µg/l)	Days
03/02/95	148.30	4	06/16/95	44.40	110
03/09/95	26.84	11	06/20/95	30.41	114
03/13/95	26.58	15	06/24/95	35.92	118
03/21/95	58.88	23	06/28/95	101.98	122
03/25/95	63.54	27	07/02/95	57.25	126
03/29/95	60.43	31	07/06/95	65.19	130
04/02/95	56.40	35	07/10/95	53.54	134
04/06/95	102.47	39	07/14/95	32.86	138
04/10/95	133.59	43	07/18/95	31.05	142
04/14/95	68.24	47	07/22/95	35.20	146
04/18/95	152.09	51	07/26/95	57.47	150
04/22/95	205.58	55	08/08/95	48.83	163
04/26/95	191.80	59	08/18/95	131.58	173
05/11/95	116.01	74	08/22/95	88.30	177
05/15/95	92.31	78	08/27/95	97.55	182
05/19/95	110.00	82	09/09/95	71.56	195
05/23/95	117.32	86	09/17/95	106.14	203
05/27/95	145.88	90	09/21/95	143.39	207
05/31/95	121.36	94	09/25/95	126.22	211
06/04/95	86.04	98	09/29/95	109.06	215
06/08/95	96.23	102	10/08/95	148.83	224
06/12/95	62.01	106	10/12/95	101.09	228

Sulfate Column 1

Date	Conc.(µg/l)	Days	Date	Conc.(µg/l)	Days
05/31/95	100.93	4	07/22/95	38.81	56
06/04/95	77.01	8	07/26/95	24.16	60
06/08/95	174.14	12	08/08/95	9.87	73
06/12/95	97.25	16	08/18/95	9.87	83
06/16/95	54.62	20	08/22/95	9.87	87
06/20/95	53.35	24	08/27/95	5.07	92
06/24/95	81.82	28	08/31/95	5.07	96
06/28/95	78.36	32	09/05/95	5.07	101
07/02/95	95.85	36	09/09/95	75.52	105
07/06/95	69.88	40	09/13/95	117.34	109
07/10/95	58.87	44	09/17/95	79.94	113
07/14/95	54.54	48	09/21/95	59.28	117
07/18/95	49.65	52	09/25/95	65.13	121

Sulfate Column 2

Date	Conc.(µg/l)	Days	Date	Conc.(µg/l)	Days
05/31/95	89.36	4	07/22/95	18.54	56
06/04/95	79.54	8	07/26/95	20.66	60
06/08/95	118.64	12	08/08/95	9.87	73
06/12/95	126.24	16	08/18/95	9.87	83
06/16/95	52.29	20	08/22/95	9.87	87
06/20/95	44.82	24	08/27/95	5.07	92
06/24/95	87.17	28	08/31/95	5.07	96
06/28/95	62.02	32	09/05/95	5.07	101
07/02/95	60.57	36	09/09/95	66.37	105
07/06/95	92.47	40	09/13/95	107.17	109
07/10/95	43.38	44	09/17/95	65.04	113
07/14/95	26.60	48	09/21/95	63.22	117
07/18/95	29.78	52	09/25/95	54.07	121

APPENDIX D
RAW DATA, ACETATE EFFLUENT CONCENTRATIONS

Aerobic Column 1

Date	Conc.(µg/l)	Days
2*/24/95	12.83	23
2*/28/95	4.26	27
3*/4/95	7.02	31
3*/11/95	10.22	38
3*/15/95	13.52	42
3*/19/95	12.88	46
3*/23/95	23.54	50
3*/27/95	23.54	54
3*/31/95	-1.70	58
4*/4/95	0.64	62
4*/8/95	8.77	66
5*/23/95	14.28	111

Aerobic Column 2

Date	Conc.(µg/l)	Days
2*/24/95	54.61	23
2*/28/95	9.55	27
3*/4/95	25.44	31
3*/11/95	14.97	38
3*/15/95	15.19	42
3*/19/95	8.15	46
3*/23/95	21.60	50
3*/27/95	21.60	54
3*/31/95	4.92	58
4*/4/95	-2.43	62
4*/8/95	4.31	66
4*/28/95	10.13	86
5*/23/95	8.17	111
7*/18/95	14.57	167
8*/22/95	12.16	202
9*/9/95	9.23	220

Aerobic Column 3

Date	Conc.(µg/l)	Days
2*/24/95	45.79	23
2*28/95	4.65	27
3*/4/95	34.31	31
3*/11/95	10.52	38
3*/15/95	10.57	42
3*/19/95	14.50	46
3*/23/95	69.83	50
3*/27/95	69.83	54
3*/31/95	3.72	58
4*/4/95	-2.77	62
4*/8/95	7.59	66
5*/23/95	13.27	111
7*/18/95	9.19	167
8*/22/95	9.19	202
9*/9/95	6.58	220

Aerobic Column 4

Date	Conc.(µg/l)	Days
2*/24/95	8.74	23
2*/28/95	10.63	27
3*/4/95	10.27	31
3*/11/95	10.96	38
3*/15/95	13.48	42
3*/19/95	16.79	46
3*/23/95	66.40	50
3*/27/95	66.40	54
3*/31/95	-3.50	58
4*/4/95	7.41	62
4*/8/95	2.28	66
5*/23/95	15.24	111
7*/18/95	-6.40	167
8*/22/95	13.16	202
9*/9/95	16.25	220

APPENDIX E
RAW DATA, HERBICIDE INFLUENT CONCENTRATIONS

Aerobic Column Influent

Propachlor (µg/l)	Alachlor (µg/l)	Day
318.20	183.00	66
286.95	231.19	90
256.30	264.00	107
368.40	312.30	131
295.60	292.10	139
	327.84	163
247.62	199.63	220
181.04	221.20	228

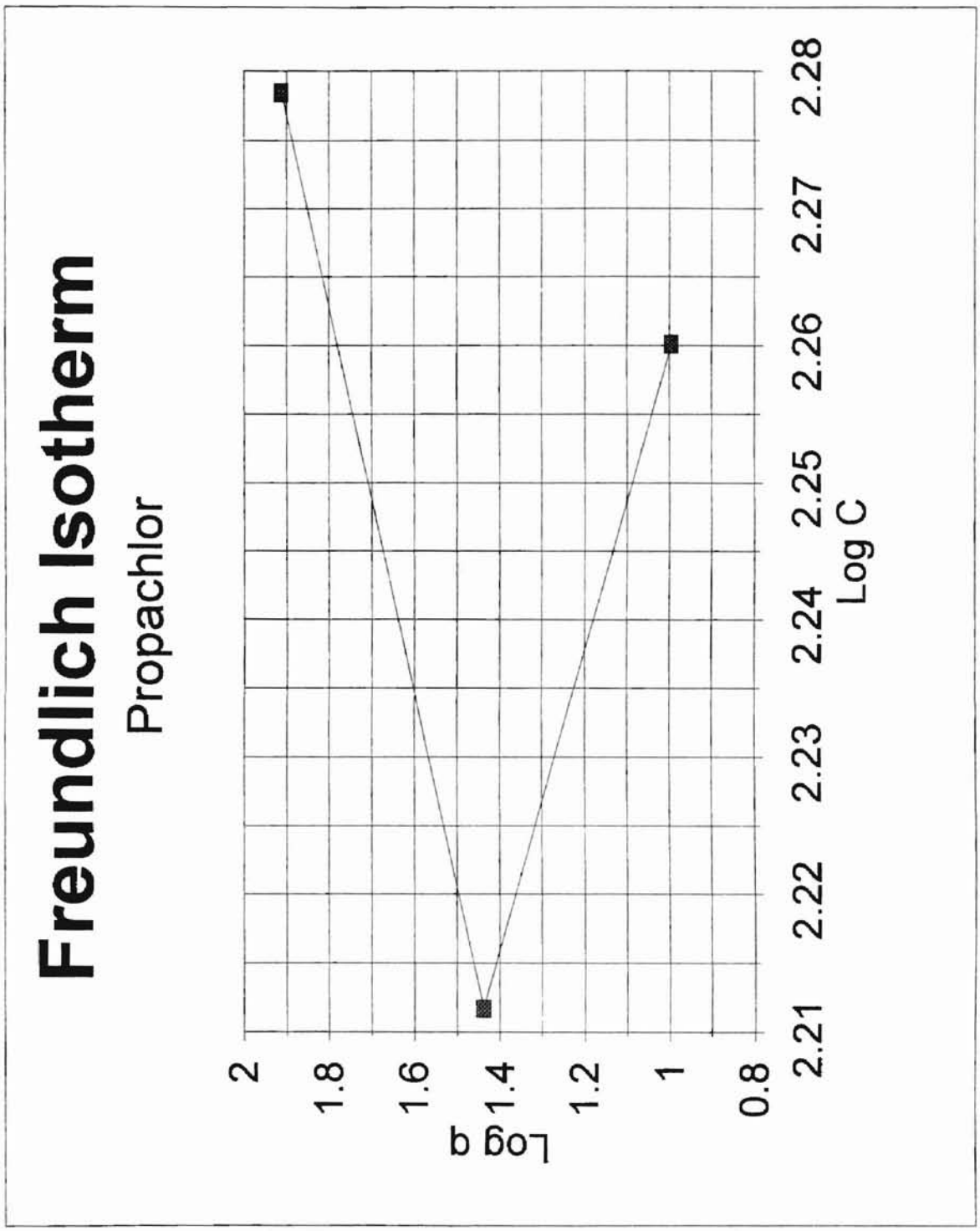
Sterile Column Influent

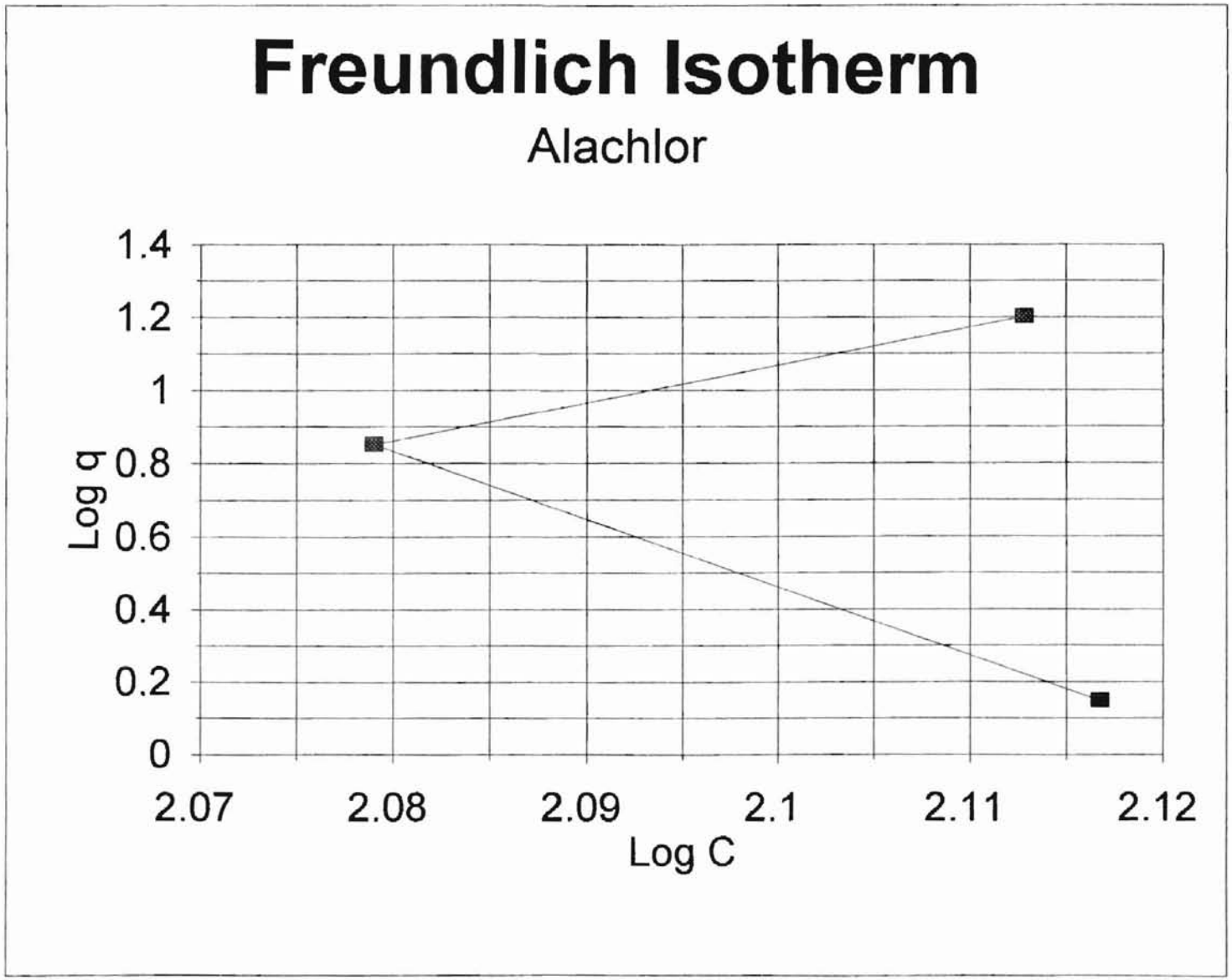
Propachlor (µg/l)	Alachlor (µg/l)	Day
318.00	223.70	41
249.40	216.30	86
252.40	182.00	118
247.62	199.63	163
181.04	221.20	203
249.76	278.11	228

Sulfate Column Influent

Propachlor (µg/l)	Alachlor (µg/l)	Day
327.90	184.10	8
290.00	264.10	24
	275.66	73
140.11	104.25	105

APPENDIX F
FREUNDLICH ISOTHERMS

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VITA 2

John R. Clink

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Master of Science

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